RESEARCH PAPER

PREPARATION AND CHARACTERIZATION OF POLYHERBAL NANOPARTICLES FOR IMPROVED ANTIDIABETIC ACTIVITY

Vijey Aanandhi Muthukumar[1], Bhoomika[2], Syed Ahmed Hussain[3], Yusupova Gulnara[4], Arkendu Chatterjee[5], Mavluda Najmetdinova[6], H Lalhlenmawia[*7], Ankita Alice Singh[*8]

[1]Professor, School of Pharmacy, SBV Chennai, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry – 607402, India, Email: mvaanandhi@gmail.com

[2]Professor, Department of Life Sciences, School of Biosciences & Technology (SBT), Galgotias University, Greater Noida (U.P.), India, Email: drbhoomika.varshney@gmail.com

[3]Professor of Pharmacotherapy and Internal Medicine, RCB College,

[4]Assistant professor, Department of hospital therapy, Email-yusupovagulnara@email.com

5 Professor, Department of Pharmacy, Sanaka Education Trust group of Institutions, Durgapur, West Bengal, Email: arkendu75@gmail.com

[6]Associate Professor, (PhD) in Philological Sciences, Department of Music Education and Culture, ORCID ID: 0000-0002-4894-9106; E-mail: Imronka@mail.ru

[7]Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Aizawl, Mizoram, Email: hlenmawia@gmail.com

[8]Lecturer, Kalaniketan Polytechnic College, Jabalpur, M.P., E-mail- ankeesingh12@gmail.com

***Corresponding author:** Dr H Lalhlenmawia, Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Aizawl, Mizoram; hlenmawia@gmail.com and Ankita Alice Singh, Lecturer, Kalaniketan Polytechnic College, Jabalpur, M.P., E-mail- ankeesingh12@gmail.com

Abstract

Background: Type 2 diabetes mellitus (T2DM) requires lifelong therapy, and single-agent drugs often fail to control hyperglycaemia and its metabolic complications. Polyherbal preparations act on several targets at once, but their polyphenols, flavonoids and saponins suffer from poor solubility, gastrointestinal degradation and low oral bioavailability. Objective: To prepare chitosan–tripolyphosphate (CS–TPP) nanoparticles loaded with a standardized polyherbal extract of *Gymnema sylvestre*, *Momordica charantia* and *Trigonella foenum-graecum*, to characterize them physicochemically, and to test whether nanoencapsulation improves antidiabetic activity. Methods: The hydroalcoholic polyherbal extract (PHE) was standardized by total phenolic and flavonoid content. Nanoparticles (PHNPs) were prepared by ionic gelation and optimized with a three-factor Box–Behnken design. The optimized batch was characterized by dynamic light scattering, zeta potential, SEM/TEM, FTIR, XRD, DSC, encapsulation efficiency, in vitro release and a 90-day stability study. Activity was assessed by α-amylase and α-glucosidase inhibition and by a 28-day study in streptozotocin–nicotinamide (STZ–NA) diabetic Wistar rats. Results: The optimized PHNPs had a mean size of [168 ± 7 nm], PDI of [0.21 ± 0.02], zeta potential of [+31.4 ± 1.8 mV] and encapsulation efficiency of [78.6 ± 2.4 %]. Spectroscopic and thermal data indicated physical entrapment and partial amorphization of the extract. Release was sustained, reaching [81 %] at 24 h. PHNPs showed lower IC_{50} values than free PHE in both enzyme assays. At half the extract dose, PHNPs lowered fasting blood glucose by [61 %] versus [38 %] for free PHE, and improved HbA1c, lipid profile, antioxidant enzymes and pancreatic histology. Conclusion: CS–TPP nanoencapsulation of a polyherbal extract improved release behaviour and antidiabetic efficacy in an STZ–NA model, supporting further pharmacokinetic and toxicological work.

Keywords: polyherbal formulation; chitosan nanoparticles; ionic gelation; antidiabetic activity; *Gymnema sylvestre*; streptozotocin–nicotinamide model; nanocharacterization

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1. Introduction

1.1 Burden of diabetes and limits of current therapy

Diabetes mellitus is now one of the largest chronic-disease burdens worldwide. The 11th edition of the IDF Diabetes Atlas estimates that 589 million adults aged 20–79 years were living with diabetes in 2024, about one in nine, and projects 853 million by 2050; roughly three in four of these adults live in low- and middle-income countries, and more than 250 million remain undiagnosed [1]. Most cases are type 2 diabetes mellitus (T2DM), characterised by insulin resistance, progressive β-cell dysfunction, chronic hyperglycaemia, dyslipidaemia and oxidative stress. Metformin, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors and GLP-1 receptor agonists achieve good glycaemic control in many patients. Long-term use, however, brings gastrointestinal intolerance, hypoglycaemia, weight change, genitourinary infections, cost, and a need for

escalating or combined regimens as β-cell function declines. These limits keep interest high in adjunct therapies that are affordable, act on several pathways, and are acceptable to patients.

1.2 Medicinal plants and the rationale for polyherbal combinations

More than a thousand plant species have been reported for diabetes management in traditional systems such as Ayurveda, Siddha, Unani and folk medicine [2,3]. *Gymnema sylvestre* (gurmar), *Momordica charantia* (bitter gourd) and *Trigonella foenum-graecum* (fenugreek) are among the most frequently studied. Gymnemic acids from *G. sylvestre* are reported to reduce intestinal glucose absorption and support β-cell function, and the plant is described as a multi-target antidiabetic agent that also modulates oxidative stress and inflammation [4]. Cucurbitane-type glycosides, charantin and an insulin receptor-binding protein from *M. charantia* have been linked to AMPK activation and increased glucose uptake in muscle cells [5]. Fenugreek seeds contribute soluble galactomannan fibre, 4-hydroxyisoleucine and saponins that slow carbohydrate digestion and enhance insulin secretion [3].

Because T2DM is a multifactorial disease, combining herbs with complementary mechanisms is rational: one component may inhibit carbohydrate-digesting enzymes while another improves peripheral glucose uptake or protects pancreatic tissue. Synergy research in phytomedicine supports the view that multi-component extracts can show effects that single constituents do not, through pharmacodynamic additivity and through improved solubility or absorption of co-constituents [6]. Polyherbal formulations tested in streptozotocin–nicotinamide (STZ–NA) rats have produced glucose-lowering effects comparable to glibenclamide and better than the single herb in some designs [13].

1.3 Why nanoformulation

Despite this promise, crude herbal extracts perform unevenly after oral dosing. Many polyphenols, flavonoids and triterpenoid saponins have low aqueous solubility, are degraded by gastric acid and intestinal enzymes, are poorly permeable, and undergo extensive first-pass metabolism. The dose needed in animals (often 250–500 mg/kg) is large, and batch-to-batch variation is high. Nanocarriers can address several of these problems at once: they protect labile constituents, increase surface area, prolong gastrointestinal residence, and allow sustained release [7,8].

Chitosan, a cationic polysaccharide obtained by deacetylation of chitin, is biocompatible, biodegradable and mucoadhesive. Under mild aqueous conditions, its protonated amino groups cross-link with polyanions such as sodium tripolyphosphate (TPP) in a process termed ionic gelation, which avoids organic solvents and high shear [9,10]. Chitosan–TPP (CS–TPP) nanoparticles have encapsulated multi-component plant extracts such as *Arrabidaea chica*, where encapsulation allowed lower doses to achieve the same gastroprotective effect as the free extract [11]. In diabetes, a combined glycyrrhizin–thymoquinone polymeric nanoformulation outperformed both single nanoformulations and the pure compounds in STZ–NA rats [12]. Controlled formulation variables such as chitosan concentration and chitosan-to-TPP ratio strongly determine particle size, surface charge and entrapment of polyphenols [10,11].

1.4 Research gap and objectives

Most nanoformulation studies in diabetes use one isolated compound or one crude extract. Systematic work that standardizes a multi-herb extract, optimizes its carrier statistically, characterizes the particles with several orthogonal techniques, and links those properties to in vitro and in vivo activity remains limited. The present study therefore aimed to:

1. prepare a standardized hydroalcoholic polyherbal extract (PHE) of *G. sylvestre* leaves, *M. charantia* fruit and *T. foenum-graecum* seeds;
2. encapsulate PHE in CS–TPP nanoparticles (PHNPs) by ionic gelation and optimize the formulation with a Box–Behnken design;
3. characterize PHNPs for size, polydispersity, zeta potential, morphology, chemical interaction, crystallinity, thermal behaviour, encapsulation efficiency, release kinetics and storage stability; and
4. compare free PHE and PHNPs for α-amylase and α-glucosidase inhibition, antioxidant capacity, and antidiabetic effects in STZ–NA diabetic rats, using metformin as reference.

2. Materials and Methods

2.1 Materials

Chitosan (medium molecular weight, 75–85 % deacetylated), sodium tripolyphosphate (TPP), streptozotocin (STZ), nicotinamide (NA), gallic acid, quercetin, acarbose, 2,2-diphenyl-1-picrylhydrazyl (DPPH), porcine pancreatic α-amylase, yeast α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG) were procured from [supplier, city, country]. Metformin hydrochloride was obtained as a gift sample from [company]. Glacial acetic acid, ethanol and other reagents were of analytical grade. Ultrapure water was used throughout. Kits for glucose, HbA1c, insulin (rat ELISA), total cholesterol, triglycerides, HDL-C and LDL-C were from [supplier].

2.2 Plant material and preparation of the polyherbal extract

Leaves of *G. sylvestre*, fruits of *M. charantia* and seeds of *T. foenum-graecum* were collected from [location] in [month, year] and authenticated by [botanist, institution]. Voucher specimens ([voucher

numbers]) were deposited in the [herbarium]. Materials were shade-dried, powdered (60 mesh) and mixed in the ratio 2:1:1 (w/w) of *G. sylvestre*, *M. charantia* and *T. foenum-graecum*. The ratio was fixed from preliminary α-glucosidase screening of binary and ternary mixtures [data not shown / supplementary]. The powder (100 g) was macerated with 70 % ethanol (1:10 w/v) for 72 h with intermittent shaking, filtered, and concentrated under reduced pressure at 45 °C using a rotary evaporator. The concentrate was freeze-dried to give the polyherbal extract (PHE), stored at 4 °C in amber glass, and the yield was recorded as a percentage of dry starting weight.

2.3 Standardization of PHE

Total phenolic content was measured by the Folin–Ciocalteu method [14] and expressed as mg gallic acid equivalents (GAE) per g extract. Total flavonoid content was measured by aluminium chloride colorimetry (510 nm) and expressed as mg quercetin equivalents (QE) per g extract. Gymnemic acid was quantified as a marker compound by HPTLC/HPLC using [method details and reference standard]. Phytochemical classes (alkaloids, flavonoids, saponins, tannins, glycosides) were screened by standard qualitative tests.

2.4 Preparation of PHE-loaded chitosan nanoparticles

PHNPs were prepared by ionic gelation [9,10]. Chitosan was dissolved in 1 % (v/v) aqueous acetic acid, stirred overnight, adjusted to pH 4.8 with 1 M NaOH and filtered (0.45 µm). PHE was dissolved in water and mixed with the chitosan solution under magnetic stirring (600 rpm) for 30 min. TPP solution was then added dropwise (1 mL/min) at 25 ± 2 °C and stirring continued for 45 min. Nanoparticles were collected by centrifugation at 15,000 × g for 30 min at 4 °C, washed twice with water, resuspended with 2 % (w/v) mannitol as cryoprotectant, and freeze-dried. Blank nanoparticles (BNPs) were prepared the same way without PHE.

2.5 Experimental design and optimization

A three-factor, three-level Box–Behnken design (17 runs including 5 centre points) was generated in [software, version] to study the effect of chitosan concentration (X1), chitosan:TPP mass ratio (X2) and PHE:chitosan ratio (X3) on particle size (Y1), polydispersity index (Y2) and encapsulation efficiency (Y3). Factor levels are shown in Table 1. Quadratic models were fitted, tested by ANOVA ($p < 0.05$, lack-of-fit not significant), and the optimum was chosen by desirability function, aiming for minimum size and PDI and maximum encapsulation efficiency. The predicted optimum was prepared in triplicate to check the prediction error.

Table 1. Factors and levels of the Box–Behnken design.

Factor	Low (−1)	Medium (0)	High (+1)
X1: Chitosan concentration (mg/mL)	1.0	2.0	3.0
X2: Chitosan:TPP (w/w)	3:1	5:1	7:1
X3: PHE:chitosan (w/w)	0.25	0.50	0.75

2.6 Physicochemical characterization

Particle size, PDI and zeta potential. Mean hydrodynamic diameter, PDI and zeta potential were measured by dynamic light scattering and electrophoretic light scattering on a [instrument, manufacturer] at 25 °C after dilution in ultrapure water (n = 3). Zeta potential beyond ±30 mV is generally taken to indicate good colloidal stability, and PDI below about 0.3 indicates a narrow distribution [15,16].

Encapsulation efficiency and loading. Free (unencapsulated) PHE in the supernatant after centrifugation was quantified as total phenolics. Encapsulation efficiency (EE, %) = (total PHE − free PHE) / total PHE × 100; drug loading (DL, %) = (total PHE − free PHE) / weight of nanoparticles × 100.

Morphology. Samples were examined by field-emission SEM ([instrument]) after gold sputtering and by TEM ([instrument]) at 200 kV after negative staining with 2 % phosphotungstic acid.

FTIR. Spectra of chitosan, TPP, PHE, BNPs, PHNPs and their physical mixture were recorded between 4000 and 400 cm^{-1} (KBr pellet or ATR, 4 cm^{-1} resolution) to assess functional-group interaction.

XRD and DSC. X-ray diffraction patterns were collected over 5–60° 2θ (Cu Kα). DSC thermograms were recorded from 30 to 300 °C at 10 °C/min under nitrogen in sealed aluminium pans.

2.7 In vitro release and kinetics

Release was studied by dialysis (12 kDa cut-off) at 37 ± 0.5 °C, 100 rpm, first in simulated gastric fluid (pH 1.2) for 2 h and then in simulated intestinal fluid (pH 6.8) up to 24 h, with sink conditions maintained. PHE solution was run as control. Phenolic content in withdrawn samples was determined at each time point (n = 3). Data were fitted to zero-order, first-order, Higuchi [17] and Korsmeyer–Peppas [18] models, and the best model chosen by the highest adjusted R^2.

2.8 Stability

Freeze-dried PHNPs were stored in sealed amber vials at 4 ± 2 °C and at 25 ± 2 °C/60 ± 5 % RH for 90 days, following ICH Q1A(R2) principles [19]. Size, PDI, zeta potential and EE were measured at 0, 30, 60 and 90 days.

2.9 In vitro antidiabetic and antioxidant assays

α-Amylase inhibition was assayed by the dinitrosalicylic acid method [20] and α-glucosidase inhibition by release of p-nitrophenol from pNPG at 405 nm. DPPH radical scavenging was measured at

517 nm [21]. PHE, PHNPs and BNPs were tested at five concentrations (n = 3) against acarbose (enzyme assays) or ascorbic acid (DPPH) as positive control, and IC_{50} values were obtained by non-linear regression. For nanoparticles, concentrations are expressed as extract equivalents.

2.10 Animals, acute toxicity and induction of diabetes

Adult male Wistar rats (180–220 g) were housed at 22 ± 2 °C, 12 h light–dark cycle, with standard pellet diet and water ad libitum. All procedures were approved by the Institutional Animal Ethics Committee ([approval no., date]) under CPCSEA guidelines. Acute oral toxicity of PHNPs was assessed following OECD guideline 423 up to 2000 mg/kg [22]. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg in cold citrate buffer, pH 4.5) given 15 min after NA (110 mg/kg), a model that yields moderate, stable hyperglycaemia with partly preserved β-cell function resembling T2DM [23]. Rats with fasting blood glucose (FBG) ≥ 200 mg/dL on day 7 were included.

2.11 Treatment protocol

Rats were randomized into seven groups (n = 6) and treated orally once daily for 28 days by gavage (Table 2). Doses for PHNPs are expressed as PHE equivalents.

Table 2. Experimental groups.

Group	Treatment	Dose (mg/kg/day, p.o.)
I	Normal control (vehicle)	—
II	Diabetic control (vehicle)	—
III	Diabetic + metformin	100
IV	Diabetic + free PHE	400
V	Diabetic + PHNPs (low)	100
VI	Diabetic + PHNPs (high)	200
VII	Normal + PHNPs (high), safety group	200

Body weight and FBG (glucometer, tail vein) were recorded weekly. An oral glucose tolerance test (OGTT, 2 g/kg glucose) was performed on day 27 after overnight fasting. On day 29, blood was collected by retro-orbital puncture under anaesthesia and serum was separated; animals were then euthanized under deep anaesthesia.

2.12 Biochemical and histopathological analysis

Serum glucose, HbA1c, insulin, total cholesterol, triglycerides, HDL-C and LDL-C were measured with commercial kits; HOMA-IR was calculated as fasting insulin (μU/mL) × FBG (mmol/L) / 22.5. Liver and pancreas homogenates were assayed for superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA). Liver glycogen was measured by the anthrone method. Pancreas, liver and kidney tissue were fixed in 10 % neutral buffered formalin, paraffin-embedded, sectioned at 5 μm and stained with haematoxylin and eosin; pancreatic islets were scored blind by a pathologist.

2.13 Statistical analysis

Data are mean ± SD (in vitro, n = 3) or mean ± SEM (in vivo, n = 6). Groups were compared by one-way ANOVA followed by Tukey's post hoc test, or two-way repeated-measures ANOVA for time-course data, in [software, version]. $p < 0.05$ was considered significant.

3. Results and Discussion

Values in [brackets] are illustrative placeholders; revise the trends described in the text to match your measured data.

3.1 Standardization of the polyherbal extract

The 70 % ethanolic extraction gave a PHE yield of [18.6 %] w/w. The extract was rich in phenolics and flavonoids and contained a measurable amount of the saponin marker gymnemic acid (Table 3). Qualitative screening was positive for flavonoids, saponins, tannins, glycosides and alkaloids [confirm against your tests]. Standardizing the extract before encapsulation is essential: the nanoparticle loading, release profile and dose equivalence all depend on a defined marker content, and without it differences between free and encapsulated extract cannot be attributed to the carrier.

Table 3. Standardization parameters of the polyherbal extract (mean ± SD, n = 3).

Parameter	Value
Extractive yield (% w/w)	[18.6 ± 0.7]
Total phenolic content (mg GAE/g extract)	[142.3 ± 5.1]
Total flavonoid content (mg QE/g extract)	[58.7 ± 2.9]
Gymnemic acid (% w/w)	[4.2 ± 0.3]

3.2 Formulation optimization

All three responses fitted quadratic models with significant model terms and non-significant lack of fit (Table 4). The high adjusted and predicted R^2 values and the small gap between them show that the models describe the design space without over-fitting.

Table 4. Statistical summary of the Box–Behnken quadratic models.

Response	R^2	Adjusted R^2	Predicted R^2	Model p	Lack of fit p
Y1: Particle size	[0.981]	[0.957]	[0.873]	[<0.0001]	[0.214]
Y2: PDI	[0.962]	[0.912]	[0.801]	[0.0004]	[0.347]
Y3: Encapsulation efficiency	[0.987]	[0.969]	[0.902]	[<0.0001]	[0.188]

The effects followed the pattern reported for CS–TPP systems [9–11]. Particle size rose with chitosan concentration because higher viscosity and polymer density favour inter-particle bridging and larger cross-linked networks. Size passed through a minimum near a chitosan:TPP ratio of 5:1: at lower ratios (more TPP) charge neutralization led to aggregation, while at higher ratios too few cross-links formed to condense the chains. Encapsulation efficiency rose with PHE:chitosan ratio up to a point, then plateaued as binding sites on the chitosan chains became saturated and surplus extract remained in the aqueous phase. The desirability function ([0.88]) selected chitosan 2.0 mg/mL, chitosan:TPP 5:1 and PHE:chitosan 0.5 as the optimum. Predicted and observed responses agreed within [±3 %] (Table 5), validating the models.

3.3 Particle size, zeta potential, entrapment and morphology

The optimized PHNPs were nanosized, narrowly distributed and positively charged (Table 5). A PDI of [0.21] indicates a fairly monodisperse population, below the 0.3 limit usually accepted for polymeric nanocarriers [16]. The zeta potential of [+31.4 mV] reflects protonated chitosan amino groups left uncrosslinked at the surface; the resulting electrostatic repulsion explains the colloidal stability, and the positive charge favours adhesion to the negatively charged intestinal mucus, which can lengthen residence time and absorption [7]. Blank nanoparticles were smaller ([121 ± 5 nm]) than PHNPs, consistent with the extract being incorporated into the polymer network rather than merely adsorbed.

Table 5. Characteristics of the optimized formulation (mean ± SD, n = 3).

Parameter	Predicted	Observed
Particle size (nm)	[165]	[168 ± 7]
PDI	[0.20]	[0.21 ± 0.02]
Zeta potential (mV)	—	[+31.4 ± 1.8]
Encapsulation efficiency (%)	[80.1]	[78.6 ± 2.4]
Drug loading (%)	—	[21.3 ± 1.1]

An encapsulation efficiency of [78.6 %] for a crude multi-component extract is satisfactory. Values reported for CS–TPP systems loaded with plant polyphenols vary widely, for example from about 26 % to 46 % in rose-hip extract nanoparticles and up to about 88 % in grape, jaboticaba and hibiscus extracts, depending on the chitosan:TPP ratio and extract concentration [24,25]. Efficiency measured by total phenolics reflects the polyphenol fraction rather than the saponin fraction, which is a limitation noted in Section 4.

SEM and TEM images (Fig. 2) showed [discrete, nearly spherical particles with smooth surfaces and little aggregation]. TEM diameters of [95–140 nm] were smaller than the DLS hydrodynamic size of [168 nm], as expected, because DLS measures the hydrated particle with its solvation layer while TEM measures the dried core.

[Insert Fig. 2: (a) SEM and (b) TEM micrographs of PHNPs, with scale bars.]

3.4 FTIR, XRD and DSC

FTIR. Chitosan showed its characteristic broad O–H/N–H stretch near 3400 cm^{-1}, amide I near 1650 cm^{-1} and N–H bending near 1590 cm^{-1} (Fig. 3). In BNPs and PHNPs the amine band shifted to [about 1540 cm^{-1}] and a new band appeared at [about 1150–1220 cm^{-1}] attributable to P=O/P–O stretching of TPP, which confirms electrostatic cross-linking of NH_3^+ groups with phosphate. PHNP spectra retained the main PHE bands (phenolic O–H, C=O and C=C aromatic bands) with reduced intensity and small shifts, indicating hydrogen-bonding or ionic interaction between extract constituents and chitosan, with no sign of new covalent bonds.

XRD. Chitosan showed broad semi-crystalline reflections near 10° and 20° 2θ, TPP showed sharp crystalline peaks, and PHE was largely amorphous (Fig. 4). PHNPs displayed a broad, low-intensity pattern with the sharp TPP peaks absent, which indicates that cross-linking disrupted chitosan chain packing and that PHE is molecularly dispersed or amorphous inside the particles. Loss of crystallinity usually increases apparent solubility and dissolution of poorly soluble compounds.

DSC. Chitosan showed a broad endotherm near [100 °C] from loss of bound water and a degradation

exotherm above [280 °C] (Fig. 5). PHNPs showed a [shifted and broadened] endotherm and a [higher] decomposition onset than the physical mixture, consistent with interaction between components and improved thermal stability.

[Insert Fig. 3: FTIR spectra. Fig. 4: XRD patterns. Fig. 5: DSC thermograms. Each shows chitosan, TPP, PHE, physical mixture, BNPs and PHNPs.]

3.5 In vitro release and kinetics

PHE dissolved and diffused freely, with [93 %] crossing the dialysis membrane within 4 h. PHNPs released the extract in a sustained, biphasic way (Table 6): a modest initial release of [22 %] in simulated gastric fluid over 2 h, then continued release in intestinal fluid to [81 %] at 24 h. The early phase probably reflects extract adsorbed near the surface, and the later phase diffusion through the swollen chitosan matrix and slow erosion. At pH 1.2 chitosan is fully protonated and TPP cross-links partly loosen, yet the retained fraction of [78 %] after 2 h shows that most of the extract is shielded from the gastric environment.

Table 6. Release of PHE from PHNPs and best-fit kinetic models (n = 3).

Parameter	PHE (free)	PHNPs
Cumulative release at 2 h (%)	[58]	[22 ± 2]
Cumulative release at 8 h (%)	[97]	[51 ± 3]
Cumulative release at 24 h (%)	[99]	[81 ± 3]
Best-fit model	—	[Korsmeyer–Peppas]
Adjusted R^2 (zero / first / Higuchi / Korsmeyer–Peppas)	—	[0.91 / 0.95 / 0.97 / 0.99]
Release exponent n	—	[0.61]

The Korsmeyer–Peppas model gave the best fit, with n between 0.43 and 0.85 for spherical particles, which points to anomalous (non-Fickian) transport combining diffusion and polymer relaxation or erosion [17,18]. [Insert Fig. 6: cumulative release profiles of PHE and PHNPs.]

3.6 Storage stability

After 90 days at 4 °C, size, PDI, zeta potential and EE changed by less than [5 %], whereas storage at 25 °C/60 % RH produced a larger drift ([8 %] size increase, [8 %] EE loss; Table 7). Phenolic leakage and slow chitosan hydrolysis at higher temperature and humidity are the likely causes. The freeze-dried product is therefore best stored refrigerated, and cryoprotectant-containing formulations should be tested under longer, ICH-aligned conditions [19].

Table 7. Stability of freeze-dried PHNPs over 90 days (mean ± SD, n = 3).

Condition	Day	Size (nm)	PDI	Zeta potential (mV)	EE (%)
4 ± 2 °C	0	[168 ± 7]	[0.21]	[+31.4]	[78.6]
4 ± 2 °C	90	[172 ± 6]	[0.22]	[+30.2]	[76.9]
25 °C / 60 % RH	0	[168 ± 7]	[0.21]	[+31.4]	[78.6]
25 °C / 60 % RH	90	[181 ± 9]	[0.26]	[+27.8]	[72.4]

3.7 In vitro enzyme inhibition and antioxidant activity

PHNPs inhibited both carbohydrate-digesting enzymes more strongly than the same amount of free PHE, shown by lower IC_{50} values (Table 8). Blank nanoparticles had negligible activity, so the effect comes from the extract and not from the carrier. The enhancement is plausible for three reasons: nanosizing gives a larger surface area for enzyme–polyphenol interaction, chitosan itself can concentrate the extract near the enzyme and protect polyphenols from oxidation during incubation, and sustained release maintains inhibitory concentrations over the assay time. PHNPs also scavenged DPPH radicals more effectively, in line with reports that encapsulation preserves phenolic antioxidant capacity [24,25].

Table 8. IC_{50} values (µg/mL of extract equivalent; mean ± SD, n = 3).

Sample	α-Amylase	α-Glucosidase	DPPH
PHE	[112.4 ± 5.8]	[86.5 ± 4.1]	[74.2 ± 3.6]
PHNPs	[68.7 ± 3.9]	[49.8 ± 2.7]	[51.3 ± 2.4]
BNPs	[> 500]	[> 500]	[> 500]
Acarbose	[46.2 ± 2.2]	[38.1 ± 1.9]	—
Ascorbic acid	—	—	[12.6 ± 0.9]

3.8 Antidiabetic activity in STZ–NA rats

Safety. No mortality or behavioural, respiratory or autonomic signs of toxicity occurred up to 2000 mg/kg PHNPs in the OECD 423 test [22], so the doses used (100 and 200 mg/kg) lie well inside the safe range [confirm with your data].

Fasting blood glucose. STZ–NA produced stable hyperglycaemia in diabetic control rats, which worsened over the 28 days (Table 9). All treatments lowered FBG, with the following order of effect at day 28: PHNPs 200 mg/kg ≈ metformin > PHNPs 100 mg/kg > free PHE 400 mg/kg > diabetic control. FBG fell by [61 %] with PHNPs 200 mg/kg and [38

%] with free PHE 400 mg/kg, so the nanoformulation matched or exceeded the free extract at half the extract dose. The dose-equivalent gain is consistent with the dose reduction reported when other plant extracts were encapsulated in chitosan [11] and with the greater efficacy of polymeric nanoformulations over pure compounds in STZ–NA rats [12].

Table 9. Fasting blood glucose (mg/dL) over 28 days (mean ± SEM, n = 6).

Group	Day 0	Day 7	Day 14	Day 21	Day 28
I Normal	[92 ± 3]	[94 ± 3]	[93 ± 2]	[95 ± 3]	[94 ± 2]
II Diabetic control	[286 ± 9]	[301 ± 10]	[318 ± 11]	[327 ± 10]	[335 ± 12]
III Metformin 100	[281 ± 8]	[215 ± 7]	[172 ± 6]	[141 ± 5]	[122 ± 5]
IV PHE 400	[284 ± 9]	[248 ± 8]	[214 ± 7]	[188 ± 7]	[176 ± 6]
V PHNPs 100	[283 ± 8]	[245 ± 8]	[205 ± 7]	[170 ± 6]	[148 ± 6]
VI PHNPs 200	[288 ± 9]	[232 ± 7]	[178 ± 6]	[138 ± 5]	[112 ± 4]

OGTT and body weight. In the OGTT, PHNP-treated rats had lower peak glucose at 30 min and returned to near baseline sooner than diabetic control and free-PHE rats, with a [34 %] smaller area under the curve for PHNPs 200 mg/kg than for free PHE (Fig. 7). Diabetic controls lost [14 %] of their body weight over 28 days from catabolic loss of protein and fat; PHNP groups recovered or maintained weight, in line with better insulin action and glycogen storage. The normal-control PHNP group (VII) showed no hypoglycaemia, so the formulation did not drive glucose below the normal range.

HbA1c, insulin and lipids. PHNPs reduced HbA1c, raised serum insulin, lowered HOMA-IR, and corrected dyslipidaemia (lower total cholesterol, triglycerides and LDL-C; higher HDL-C) more than free PHE (Table 10). HbA1c reflects glycaemia over the preceding weeks, so its fall supports a sustained effect and not a transient post-dose one. The rise in insulin together with lower HOMA-IR suggests combined β-cell protection and better insulin sensitivity, which fits the proposed actions of gymnemic acids and *M. charantia* constituents on β-cells and AMPK-mediated glucose uptake [4,5].

Table 10. Biochemical parameters at day 28 (mean ± SEM, n = 6).

Parameter	I Normal	II Diabetic	III Metformin	IV PHE 400	V PHNPs 100	VI PHNPs 200
HbA1c (%)	[4.6]	[9.4]	[6.4]	[7.6]	[7.0]	[6.1]
Insulin (µU/mL)	[16.5]	[8.2]	[13.1]	[11.0]	[12.2]	[14.3]
HOMA-IR	[3.8]	[6.8]	[4.0]	[4.8]	[4.5]	[4.0]
Total cholesterol (mg/dL)	[74]	[142]	[96]	[118]	[106]	[91]
Triglycerides (mg/dL)	[68]	[156]	[92]	[121]	[104]	[88]
HDL-C (mg/dL)	[42]	[24]	[37]	[31]	[34]	[39]
LDL-C (mg/dL)	[22]	[86]	[41]	[64]	[52]	[38]
SOD (U/mg protein)	[12.4]	[5.8]	[9.9]	[8.3]	[9.1]	[10.6]
CAT (U/mg protein)	[38.5]	[17.2]	[31.4]	[25.7]	[28.9]	[33.1]
GSH (µmol/g tissue)	[6.8]	[3.1]	[5.6]	[4.4]	[5.0]	[5.9]
MDA (nmol/mg protein)	[1.6]	[4.9]	[2.4]	[3.5]	[3.0]	[2.2]
Liver glycogen (mg/g)	[38]	[14]	[31]	[24]	[28]	[33]

Oxidative stress and histology. STZ generates reactive oxygen species and nitrosative stress in β-cells, so antioxidant status is central to protection. PHNPs restored SOD, CAT and GSH and lowered MDA toward normal values (Table 10), consistent with the stronger DPPH scavenging in vitro. Histology (Fig. 8) showed [islet atrophy, reduced islet number and vacuolated β-cells in diabetic controls; partial restoration of islet size and cell density with free PHE; and near-normal islet

architecture with PHNPs 200 mg/kg]. Liver sections showed [reduced steatosis and hepatocyte vacuolation] and kidney sections [preserved tubules and glomeruli] in treated groups [replace with your pathologist's findings].

[Insert Fig. 7: OGTT curves and AUC. Fig. 8: H&E sections of pancreas (×400), liver and kidney for groups I–VI.]

3.9 Integrated interpretation

The in vitro and in vivo results converge on a coherent picture. Ionic gelation produced stable, positively charged nanoparticles in which the extract is held by hydrogen bonding and electrostatic interaction inside a partly amorphous matrix. That structure shields phenolics and saponins from gastric conditions, releases them gradually in the intestine, and keeps their enzyme-inhibiting and antioxidant capacity higher than the free extract. In rats, the same dose-sparing effect appeared as better control of fasting glucose, HbA1c, lipids and oxidative markers, probably through a combination of reduced carbohydrate digestion, protection of β-cells and improved glucose uptake. Whether higher bioavailability or simply longer exposure explains the gain cannot be settled from these data, because plasma pharmacokinetics of marker compounds were not measured.

Two cautions apply when comparing with the literature. First, the STZ–NA model produces partial β-cell loss with limited insulin resistance, whereas the high-fat-diet/low-dose STZ model reproduces the insulin-resistant phenotype of human T2DM more faithfully [26]; confirming the effect in the second model would strengthen translational relevance. Second, different polyherbal and nanoformulation studies use different doses, durations and reference drugs, so effect sizes should be compared qualitatively, not numerically [12,13].

4. Conclusion

A standardized polyherbal extract of *G. sylvestre*, *M. charantia* and *T. foenum-graecum* was encapsulated in chitosan–TPP nanoparticles by a mild ionic gelation process and optimized with a Box–Behnken design. The optimized particles were nanosized, narrowly distributed, positively charged and physically stable under refrigeration. FTIR, XRD and DSC indicated that the extract is held by non-covalent interaction in a partly amorphous matrix, and release was sustained and followed anomalous transport kinetics. Compared with the free extract, the nanoparticles inhibited α-amylase and α-glucosidase more strongly and, in STZ–NA diabetic rats, achieved a larger fall in fasting glucose and HbA1c, better lipid and antioxidant profiles and better islet preservation at half the extract dose. Chitosan–TPP nanoencapsulation is therefore a simple and promising way to raise the potency of polyherbal antidiabetic preparations.

4.1 Limitations and future work

- Entrapment was quantified through total phenolics; marker-specific assays (gymnemic acid, charantin, 4-hydroxyisoleucine) by HPLC or LC–MS would show whether the saponin and peptide fractions are encapsulated as efficiently.
- No plasma pharmacokinetics or tissue distribution were measured, so improved bioavailability is inferred and not demonstrated.
- A single animal model, one sex, one dose range and a 28-day duration were used. Replication in a high-fat-diet/STZ model, with both sexes, longer dosing and sub-chronic toxicity testing (OECD 407), is needed.
- Mechanistic readouts (GLUT4, AMPK, PI3K/Akt, insulin-signalling expression) and permeability studies (Caco-2, everted sac) would link particle properties to the pathways proposed.
- Scale-up, batch reproducibility, and long-term stability under ICH conditions must be shown before any translational claim.

References

1. International Diabetes Federation. IDF Diabetes Atlas, 11th edition. Brussels: International Diabetes Federation; 2025. https://diabetesatlas.org
2. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol. 2002;81(1):81–100.
3. Aumeeruddy MZ, Mahomoodally MF. Ethnomedicinal plants for the management of diabetes worldwide: a systematic review. Curr Med Chem. 2021;28(23):4670–4693. doi:10.2174/0929867328666210121123037
4. Ziyanok-Demirtas S, Serin I. Gymnema sylvestre as a multi-target antidiabetic agent: mechanistic insights and metabolic regulation. Int J Mol Sci. 2026;27(12):5609. doi:10.3390/ijms27125609
5. Liu Z, Gong J, Huang W, Lu F, Dong H. The effect of Momordica charantia in the treatment of diabetes mellitus: a review. Evid Based Complement Alternat Med. 2021;2021:3796265. doi:10.1155/2021/3796265
6. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine. 2009;16(2–3):97–110.
7. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnology. 2018;16:71.

8. Mohanraj VJ, Chen Y. Nanoparticles – a review. Trop J Pharm Res. 2006;5(1):561–573.
9. Calvo P, Remuñán-López C, Vila-Jato JL, Alonso MJ. Novel hydrophilic chitosan–polyethylene oxide nanoparticles as protein carriers. J Appl Polym Sci. 1997;63(1):125–132.
10. Fan W, Yan W, Xu Z, Ni H. Formation mechanism of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique. Colloids Surf B Biointerfaces. 2012;90:21–27.
11. [Authors]. Chitosan–tripolyphosphate nanoparticles as Arrabidaea chica standardized extract carrier: synthesis, characterization, biocompatibility, and antiulcerogenic activity. Int J Nanomedicine. 2015;10. doi:10.2147/IJN.S83705 [complete authors and pages]
12. [Authors]. Antidiabetic activity enhancement in streptozotocin + nicotinamide–induced diabetic rats through combinational polymeric nanoformulation. Int J Nanomedicine. 2019;14. PMC6580421 [complete authors, pages, doi]
13. [Authors]. Antidiabetic activity of polyherbal formulation in streptozotocin–nicotinamide induced diabetic Wistar rats. J Tradit Complement Med. 2014. PMC4003700 [complete authors, volume, pages]
14. Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144–158.
15. Clogston JD, Patri AK. Zeta potential measurement. Methods Mol Biol. 2011;697:63–70.
16. Danaei M, Dehghankhold M, Ataei S, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):57.
17. Higuchi T. Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci. 1963;52(12):1145–1149.
18. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm. 1983;15(1):25–35.
19. ICH. Q1A(R2): Stability testing of new drug substances and products. Geneva: International Council for Harmonisation; 2003.
20. Bernfeld P. Amylases, α and β. Methods Enzymol. 1955;1:149–158.
21. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT – Food Sci Technol. 1995;28(1):25–30.
22. OECD. Test No. 423: Acute oral toxicity – acute toxic class method. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2002.
23. Masiello P, Broca C, Gross R, et al. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes. 1998;47(2):224–229.
24. Stoica R, Somoghi R, Ion RM. Preparation of chitosan–tripolyphosphate nanoparticles for the encapsulation of polyphenols extracted from rose hips. Dig J Nanomater Biostruct. 2013;8(3):955–963.
25. Oliveira MOS. Nanoencapsulation of bioactive extracts from grape, jaboticaba, and hibiscus by-products using chitosan–TPP: characterization, bioactive properties, and application in cassava-based foods. Universidade Federal de Goiás institutional repository [year to confirm].
26. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52(4):313–320.