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Tinospora crispa Phytosome Enhances Oral Bioavailability and Glycemic Control in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus remains a major global health challenge with rising prevalence in Southeast Asia, where traditional herbal remedies continue to play a significant role in disease management. *Tinospora crispa* (L.) Hook. f. & Thomson (Menispermaceae), known locally as *brotowali* in Indonesian *jamu* medicine, exhibits anti-diabetic properties attributed to its alkaloid and diterpenoid constituents; however, the oral bioavailability of its key bioactive compound berberine remains below 5%.

Objective: To evaluate the pharmacokinetic enhancement and anti-diabetic efficacy of a *Tinospora crispa* phytosome in streptozotocin (STZ)-induced diabetic rats.

Methods: Thirty male Wistar rats were allocated to five groups (n = 6): normal control, diabetic control, diabetic plus metformin (200 mg/kg), diabetic plus *T. crispa* free extract (400 mg/kg), and diabetic plus *T. crispa* phytosome (400 mg/kg), given orally for 28 days; a separate single-dose pharmacokinetic study used 12 healthy rats.

Results: The phytosome achieved a 3.14-fold enhancement in relative oral bioavailability (AUC_{0-24}: 1524.7 ± 185.4 versus 486.3 ± 62.8 ng·h/mL, $p < 0.001$) and a higher peak plasma berberine concentration (C_{max}: 387.2 ± 42.3 versus 124.5 ± 18.7 ng/mL, $p < 0.001$). After 28 days, the phytosome group showed reductions in fasting blood glucose (148.6 ± 19.2 versus 328.4 ± 42.5 mg/dL, $p < 0.001$) and HbA_{1c} (6.1 ± 0.6 versus 9.2 ± 1.1%, $p < 0.001$), with an improved lipid profile comparable to metformin and large effect sizes (Cohen's d: 3.51–6.22).

Conclusion: Phytosome technology increases the bioavailability and anti-diabetic efficacy of *T. crispa*, supporting its development as a standardized herbal complementary therapy for diabetes mellitus.

All authors have reviewed and approved the final version of the manuscript.

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1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both, constituting one of the most pressing global health challenges of the twenty-first century. According to the International Diabetes Federation (IDF) Diabetes Atlas, the global prevalence of diabetes reached approximately 537 million adults (20–79 years) in 2021, with projections of 643 million by 2030 and 783 million by 2045.[1] The Southeast Asian region bears a disproportionate burden, with Indonesia among the largest diabetic populations globally, harboring an estimated 19.5 million adults with diabetes.[1] The economic burden is compounded by high rates of microvascular and macrovascular complications — diabetic nephropathy, retinopathy, neuropathy, and cardiovascular disease — which account for significant morbidity, disability, and premature mortality.[2]

The pathophysiology of type 2 diabetes mellitus (T2DM), which constitutes approximately 90–95% of

all cases, involves insulin resistance in peripheral tissues, progressive pancreatic beta-cell dysfunction, excessive hepatic glucose output, and chronic low-grade inflammation.[2] At the molecular level, insulin resistance is characterized by impaired insulin receptor substrate (IRS) phosphorylation, reduced phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling, and diminished glucose transporter type 4 (GLUT4) translocation, while glucotoxicity and lipotoxicity induce oxidative stress and accelerate beta-cell apoptosis. Conventional management relies on metformin as first-line therapy, supplemented by sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists; however, these agents carry adverse effects and long-term costs that limit adherence in resource-limited settings across Indonesia and Southeast Asia.[3]

These limitations have stimulated interest in complementary plant-derived anti-diabetic agents with multi-target activity. *Tinospora crispa* (L.) Hook. f. & Thomson (Menispermaceae), a climbing shrub widely distributed across tropical Southeast Asia, has been used for centuries in Indonesian traditional medicine (*jamu*), where the stem — known locally as "*brotowali*" — serves as a bitter tonic for diabetes ("*kencing manis*"), fever, malaria, and rheumatic conditions.[4] Its ethnobotanical documentation across multiple Southeast Asian cultures provides robust empirical support for its therapeutic potential in metabolic disorders.[4]

Phytochemical investigations of *T. crispa* stem reveal a rich composition of bioactive secondary metabolites, including the protoberberine alkaloids berberine, palmatine, magnoflorine, and jatrorrhizine (berberine 1.5–3.2% of dry weight), the clerodane diterpenoids borapetoside C and tinocrisposide, and various flavonoids and phenolic acids.[4] Berberine activates the adenosine monophosphate-activated protein kinase (AMPK) pathway through inhibition of mitochondrial complex I, promoting GLUT4 translocation, enhancing fatty-acid oxidation, and suppressing hepatic gluconeogenesis.[5] In addition, network-pharmacology and *in vitro* metabolomic studies show that *T. crispa* diterpenoids and minor constituents enhance insulin sensitivity through IRS-1/PI3K/Akt signaling and GLUT4 translocation, complementary to berberine's predominantly insulin-independent action.[6,7]

Despite these attributes, clinical translation of *T. crispa* therapy has been constrained by the poor oral bioavailability of berberine — below 5% — attributable to limited intestinal absorption, P-glycoprotein-mediated efflux, extensive first-pass metabolism by CYP2D6, CYP3A4, and CYP1A2, and self-aggregation in gastrointestinal fluids.[8] These pharmacokinetic barriers necessitate innovative formulation strategies.

Phytosome technology, also termed phyto-phospholipid complexation, enhances the oral bioavailability of poorly absorbed phytoconstituents. Unlike conventional liposomes, phytosomes form a stoichiometric molecular complex between the phytoconstituent and a phospholipid (typically soy phosphatidylcholine), yielding an amphiphilic unit cell with enhanced membrane permeability, gastrointestinal protection, and lymphatic absorption.[9] Phytosomes have shown two- to five-fold bioavailability enhancement for diverse phytochemicals, including polyphenol-enriched fractions,[10] curcumin,[11] and silybin,[12] and berberine phytosome itself has advanced to controlled clinical evaluation.[13] To date, however, no phytosome formulation of *T. crispa* extract has been reported, and its pharmacokinetic–pharmacodynamic relationship in a diabetic model remains unexplored.

This study therefore aimed to develop and characterize a phytosome formulation of *T. crispa* stem extract, evaluate its pharmacokinetic enhancement of berberine oral bioavailability, and assess its anti-diabetic efficacy in an STZ-induced diabetic rat model with comprehensive metabolic endpoints — glycemic control, lipid-profile modulation, and pancreatic histopathological protection — in direct comparison with free *T. crispa* extract and a metformin positive control.

2. Methods

2.1. Study design and ethical approval

This randomized controlled experimental study was conducted at the Pharmacology Research Laboratory of a private university in Palembang, Indonesia, between February and August 2024. It comprised (a) a single-dose pharmacokinetic study comparing *T. crispa* phytosome versus free extract, and (b) a 28-day anti-diabetic efficacy study in STZ-induced diabetic rats. The protocol was reviewed and approved by the CMHC Ethics Committee (Approval No. 2024/0198; approved 12 January 2024) in accordance with the Declaration of Helsinki, the National Guidelines for Health Research Ethics of the Republic of Indonesia, and the ARRIVE guidelines version 2.0. Treatment allocation used computer-generated randomization by an independent statistician, with blinding of outcome assessors. The study was designed with a statistical power of 0.80, $\alpha = 0.05$, and an anticipated large effect

size (f = 0.40). In total, 42 male Wistar rats were used: 30 in the 28-day efficacy study and 12 in the single-dose pharmacokinetic study.

2.2. Plant material, authentication, and extraction

Fresh stems of *T. crispa* (Menispermaceae) were collected from a cultivated medicinal-plant garden in the South Sumatra region of Indonesia (tropical monsoon climate, Köppen Af) during the wet season (February 2024), when alkaloid content is maximal. Botanical authentication was performed by a plant taxonomist, and a voucher specimen (TC-2024-038) was deposited at the university herbarium. HPTLC on silica gel 60 F_{254} plates confirmed identity against the reference *T. crispa* profile and a berberine standard (R_f = 0.45).

Stems were sliced, dried (Memmert UF110, Germany) at 45 °C for 72 h to below 10% moisture, pulverized, and sieved through a 60-mesh (250 µm) screen. Extraction used maceration with 70% ethanol (1:10 w/v) for 72 h at room temperature (28 ± 2 °C), followed by vacuum filtration and rotary evaporation at 40 °C (Büchi R-210, Switzerland). Crude yield was 12.4% (w/w). HPLC (Shimadzu LC-20AD; C18; acetonitrile–0.1% phosphoric acid 30:70 v/v; 346 nm) confirmed berberine 2.47% (w/w) and palmatine 0.86% (w/w).

2.3. Phytosome preparation and characterization

The phytosome was prepared by solvent evaporation. The standardized extract and soy phosphatidylcholine (Lipoid S100, Germany) were dissolved in a 1:2 molar ratio (berberine:phosphatidylcholine) in anhydrous tetrahydrofuran at 40 °C under nitrogen for 2 h. The solvent was evaporated under reduced pressure; the film was vacuum-dried, hydrated, and lyophilized (Labconco FreeZone 6, USA) with 5% (w/v) mannitol as cryoprotectant. Three independent batches were prepared.

Characterization included particle size, polydispersity index (PDI), and zeta potential by dynamic light scattering (Zetasizer Nano ZS90, UK); complexation efficiency, CE (%) = [(Total berberine − Free berberine)/Total berberine] × 100; differential scanning calorimetry; FTIR (4000–400 cm^{-1}); and scanning electron microscopy. The optimized phytosome had a mean particle size of 186.3 ± 12.5 nm, PDI 0.245 ± 0.04, zeta potential −32.4 ± 2.8 mV, and complexation efficiency 91.7 ± 2.6% (inter-batch CV < 5.2%). DSC showed loss of the berberine endotherm at 145 °C and a new broad endotherm at 168 °C, while FTIR showed a P=O stretching shift from 1236 to 1228 cm^{-1}, confirming hydrogen bonding.

2.4. Animals, diabetes induction, and groups

Thirty male Wistar rats (*Rattus norvegicus*), aged 10–12 weeks and weighing 200–240 g, were obtained from an institutional breeding facility and housed under standard conditions (22 ± 2 °C, 55 ± 10% humidity, 12-h light/dark cycle) with free access to standard chow (AIN-93M) and purified water, after a 7-day acclimatization period.

Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg; Sigma-Aldrich) freshly dissolved in ice-cold 0.1 M citrate buffer (pH 4.5) after a 12-h fast.[14] Normal controls received citrate buffer. Tail-vein glucose was measured 72 h later (Accu-Chek Performa, Roche); only rats with fasting blood glucose (FBG) exceeding 250 mg/dL entered the diabetic groups (100%, 24/24). Rats were randomized to five groups (n = 6 each): normal control (NC; buffer plus 0.5% carboxymethylcellulose [CMC]); diabetic control (DC; STZ plus vehicle); DC plus metformin hydrochloride 200 mg/kg; DC plus *T. crispa* free extract 400 mg/kg; and DC plus *T. crispa* phytosome 400 mg/kg extract-equivalent. All treatments were given by oral gavage once daily for 28 days.

2.5. Pharmacokinetic study

A separate single-dose study used 12 healthy male Wistar rats in two groups (n = 6): free extract (400 mg/kg) or phytosome (equivalent) by gavage after a 12-h fast. Serial blood (≈200 µL) was collected from the retro-orbital plexus under brief isoflurane anesthesia at pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h. Plasma berberine was quantified by validated LC-MS/MS (Shimadzu LCMS-8060; positive electrospray; palmatine-d3 internal standard; $r^2 > 0.999$, 1–1000 ng/mL; CV < 8.5%; accuracy 95.2–104.7%). Parameters (C_{max}, T_{max}, $AUC_{0–24}$, $AUC_{0–\infty}$, $t_{1/2}$, MRT) were derived by non-compartmental analysis (PKSolver v2.0); relative bioavailability F_{rel} = ($AUC_{phytosome}$ / $AUC_{free\ extract}$) × 100%.

2.6. Anti-diabetic efficacy assessment

Body weight and FBG were monitored weekly. At Day 28, after a 12-h fast, rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and euthanized by cardiac exsanguination per AVMA guidelines. Outcomes included FBG (glucose oxidase), glycated hemoglobin (HbA_{1c}; boronate-affinity chromatography), fasting insulin (rat-specific ELISA, Mercodia), HOMA-IR = [fasting insulin (µIU/mL) × fasting glucose (mg/dL)] / 405, and a full lipid profile (total cholesterol [TC], triglycerides [TG], LDL-C, HDL-C). For histopathology, pancreata were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned at 4 µm, and stained with hematoxylin and

eosin; two blinded pathologists (Cohen's κ = 0.87) scored islets from 0 (normal) to 4 (near-complete beta-cell destruction).

2.7. Statistical analysis

Data are mean ± standard deviation (SD); analyses used SPSS v26.0 (IBM). Normality (Shapiro–Wilk) and homogeneity of variance (Levene) were confirmed (all $p > 0.05$). One-way ANOVA with partial eta-squared (η^2) was used for between-group comparisons, followed by Tukey's HSD; the pharmacokinetic study used an independent-samples t-test. Pairwise effect sizes used Cohen's d, with Bonferroni correction across outcomes. A two-tailed $p < 0.05$ was significant. Sample size was determined a priori (G*Power v3.1.9.7; one-way ANOVA, 5 groups, f = 0.40, α = 0.05, power = 0.80; minimum total n = 25).

3. Results

3.1. Animal characteristics and baseline parameters

Baseline characteristics of the experimental animals are presented in Table 1. There were no significant differences among groups in body weight ($F(4,25) = 0.08$, $p = 0.973$, $\eta^2 = 0.013$), age ($p = 0.952$), or baseline FBG ($p = 0.987$), confirming successful randomization. After STZ induction, all diabetic groups developed stable hyperglycemia (FBG exceeding 330 mg/dL) by Day 3, with no differences among the four diabetic groups ($p = 0.982$), whereas NC maintained euglycemia (93.8 ± 7.5 mg/dL, $p < 0.001$ versus all diabetic groups). All thirty rats survived the 28-day period.

Table 1. Baseline characteristics of experimental animals.

Parameter	NC	DC	DC+Met	DC+Extract	DC+Phyto	p-value
Body weight (g)	218.4 ± 12.3	215.8 ± 11.5	216.2 ± 10.8	217.1 ± 9.7	214.5 ± 13.2	0.973
Age (weeks)	11.2 ± 0.8	11.1 ± 0.9	11.3 ± 0.7	11.0 ± 0.8	11.2 ± 0.9	0.952
Baseline FBG (mg/dL)	93.8 ± 7.5	95.2 ± 8.1	94.6 ± 7.3	96.1 ± 8.7	95.8 ± 7.9	0.987

Data are mean ± SD (n = 6 per group); p-values from one-way ANOVA. NC, normal control; DC, diabetic control; Met, metformin; Phyto, phytosome; FBG, fasting blood glucose.

3.2. Pharmacokinetic enhancement by the phytosome

The plasma concentration–time profiles of berberine are illustrated in Figure 1, and the corresponding parameters are summarized in Table 2. The phytosome produced higher values for every parameter measured. Peak plasma concentration (C_{max}) was 3.11-fold higher in the phytosome group (387.2 ± 42.3 ng/mL) than the free extract (124.5 ± 18.7 ng/mL, $p < 0.001$, Cohen's d = 8.05), and time to peak (T_{max}) was shorter (1.8 ± 0.3 versus 2.5 ± 0.4 h, $p = 0.003$). The $AUC_{0–24}$ was 3.14-fold greater for the phytosome (1524.7 ± 185.4 versus 486.3 ± 62.8 ng·h/mL, $p < 0.001$), yielding a relative oral bioavailability of 314.2%. The elimination half-life ($t_{1/2}$: 5.4 ± 0.7 versus 3.8 ± 0.6 h) and mean residence time (MRT: 7.8 ± 0.9 versus 5.2 ± 0.8 h) were both prolonged ($p < 0.001$), indicating sustained systemic exposure.

Table 2. Pharmacokinetic parameters of berberine following single-dose oral administration.

Parameter	Free Extract	Phytosome	p-value
C_{max} (ng/mL)	124.5 ± 18.7	387.2 ± 42.3	< 0.001
T_{max} (h)	2.5 ± 0.4	1.8 ± 0.3	0.003
$AUC_{0–24}$ (ng·h/mL)	486.3 ± 62.8	1524.7 ± 185.4	< 0.001
$AUC_{0–\infty}$ (ng·h/mL)	512.8 ± 74.2	1687.4 ± 201.3	< 0.001
$t_{1/2}$ (h)	3.8 ± 0.6	5.4 ± 0.7	< 0.001
MRT (h)	5.2 ± 0.8	7.8 ± 0.9	< 0.001
Relative bioavailability (%)	100	314.2 ± 28.6	—

Data are mean ± SD (n = 6 per group); p-values from independent-samples t-test. C_{max}, peak plasma concentration; T_{max}, time to peak; AUC, area under the concentration–time curve; $t_{1/2}$, elimination half-life; MRT, mean residence time.

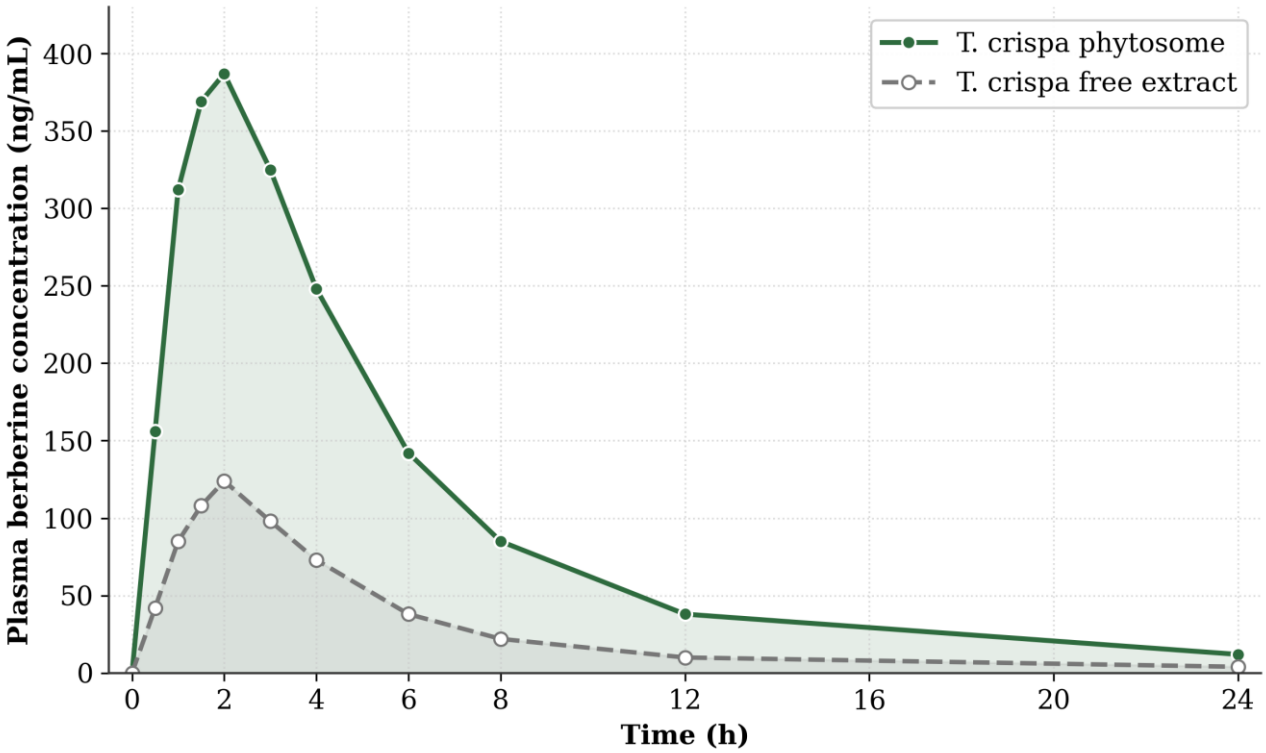


Figure 1. Plasma berberine concentration–time profiles following a single oral dose of *T. crispa* free extract and phytosome formulation. Values are mean ± SD (n = 6 per group); shaded areas represent the area under the curve.

3.3. Effects on glycemic parameters

The effects of 28-day treatment on glycemic parameters are presented in Table 3 and Figure 2. Diabetic control rats exhibited persistent severe hyperglycemia (FBG 328.4 ± 42.5 mg/dL at Day 28). The phytosome reduced FBG to 148.6 ± 19.2 mg/dL (a 54.8% reduction versus DC; mean difference −179.8 mg/dL, 95% CI −221.3 to −138.3, $p < 0.001$, Cohen's d = 5.44), comparable to metformin (142.8 ± 18.7 mg/dL, 56.5%, $p = 0.891$ versus phytosome). The free extract achieved a more modest reduction to 198.5 ± 24.3 mg/dL (39.6%), significantly inferior to both phytosome and metformin (both $p < 0.001$).

HbA_{1c} followed a concordant pattern: the phytosome (6.1 ± 0.6%) showed a 33.7% reduction relative to DC (9.2 ± 1.1%), approaching metformin (5.9 ± 0.5%, 35.9%), while the free extract achieved 22.8% (7.1 ± 0.8%). Fasting insulin was significantly restored in the phytosome group (14.2 ± 2.0 µIU/mL) versus DC (6.2 ± 1.3 µIU/mL, $p < 0.001$), approaching metformin (14.8 ± 2.1) and NC (18.5 ± 2.4 µIU/mL), as detailed in Table 3.

Table 3. Effects of 28-day treatment on glycemic parameters.

Parameter	NC	DC	DC+Met	DC+Extract	DC+Phyto	p-value
FBG (mg/dL)	95.2 ± 8.3	328.4 ± 42.5	142.8 ± 18.7	198.5 ± 24.3	148.6 ± 19.2	< 0.001
HbA_{1c} (%)	5.2 ± 0.4	9.2 ± 1.1	5.9 ± 0.5	7.1 ± 0.8	6.1 ± 0.6	< 0.001
Fasting insulin (µIU/mL)	18.5 ± 2.4	6.2 ± 1.3	14.8 ± 2.1	10.3 ± 1.8	14.2 ± 2.0	< 0.001
HOMA-IR	2.2 ± 0.3	5.1 ± 0.9	2.6 ± 0.4	4.0 ± 0.7	2.8 ± 0.5	0.214

Data are mean ± SD (n = 6 per group); p-values from one-way ANOVA. HOMA-IR, homeostatic model assessment of insulin resistance.

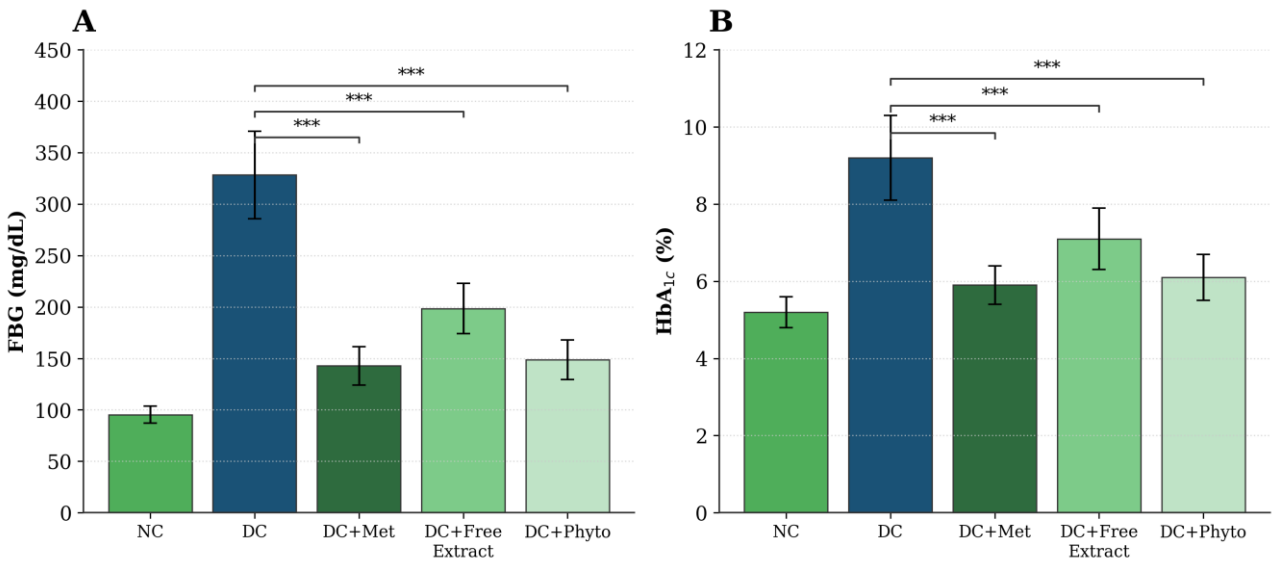


Figure 2. Glycemic parameters across treatment groups after 28 days. (A) Fasting blood glucose; (B) HbA_{1c}. Bars are mean ± SD (n = 6 per group). ***p < 0.001 versus diabetic control.

3.4. Effects on lipid profile parameters

Diabetic control rats developed characteristic dyslipidemia with elevated TC, TG, and LDL-C and reduced HDL-C versus NC (all $p < 0.001$), as summarized in Table 4. The phytosome produced lower serum lipid concentrations: TC fell to 102.7 ± 13.5 mg/dL (39.1% reduction), TG to 86.4 ± 11.2 mg/dL (53.4%), and LDL-C to 45.2 ± 8.1 mg/dL (54.1%), while HDL-C rose to 44.8 ± 5.9 mg/dL (a 57.2% increase) (all $p < 0.001$). These were comparable to metformin (TC $p = 0.872$; TG $p = 0.785$; LDL-C $p = 0.831$; HDL-C $p = 0.789$), whereas the free extract was intermediate and significantly inferior to the phytosome for every parameter (all $p < 0.01$).

Table 4. Effects of 28-day treatment on serum lipid profile (mg/dL).

Parameter	NC	DC	DC+Met	DC+Extract	DC+Phyto	p-value
Total cholesterol	78.5 ± 9.2	168.7 ± 22.3	99.4 ± 12.8	128.6 ± 16.4	102.7 ± 13.5	< 0.001
Triglycerides	72.3 ± 8.9	185.6 ± 25.4	82.1 ± 10.5	124.7 ± 15.8	86.4 ± 11.2	< 0.001
LDL-C	32.1 ± 5.3	98.4 ± 14.7	42.8 ± 7.4	67.5 ± 10.2	45.2 ± 8.1	< 0.001
HDL-C	52.4 ± 5.1	28.5 ± 4.8	46.2 ± 5.5	37.8 ± 5.2	44.8 ± 5.9	< 0.001

Data are mean ± SD (n = 6 per group); p-values from one-way ANOVA. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

3.5. Pancreatic histopathology and beta-cell protection

The diabetic control group exhibited severe islet damage with extensive beta-cell destruction, islet shrinkage, vacuolar degeneration, and inflammatory infiltration (score 3.5 ± 0.4). The phytosome group demonstrated significant protection (score 1.3 ± 0.3; 62.9% improvement versus DC, Cohen's $d = 6.22$, $p < 0.001$), comparable to metformin (1.2 ± 0.3, $p = 0.923$), while the free extract showed moderate improvement (2.1 ± 0.4; 40.0%, $p < 0.001$ versus DC).

3.6. Effect sizes and analysis of variance

Effect-size analysis (Table 5) confirmed large effects across all primary and secondary outcomes for the phytosome versus diabetic-control comparison. Cohen's d ranged from 3.51 (HbA_{1c}) to 6.22 (histopathology), and the forest plot (Figure 3) visualizes the consistent benefit across every metabolic parameter. The ANOVA partial η^2 values ranged from 0.87 to 0.95 for primary outcomes, confirming that group assignment explained most of the variance.

Table 5. Effect sizes and analysis of variance for metabolic outcomes (diabetic control versus phytosome).

Outcome	Cohen's d	95% CI	Partial η^2
Fasting blood glucose	5.44	4.12 to 6.76	0.89
HbA_{1c}	3.51	2.34 to 4.68	0.87
Total cholesterol	3.78	2.55 to 5.01	0.88
Triglycerides	4.12	2.87 to 5.37	0.91
LDL-C	4.26	3.01 to 5.51	0.92
HDL-C	3.95	2.72 to 5.18	0.90
Histopathology score	6.22	4.89 to 7.55	0.95

Cohen's d with 95% confidence intervals and ANOVA partial η^2. CI, confidence interval.

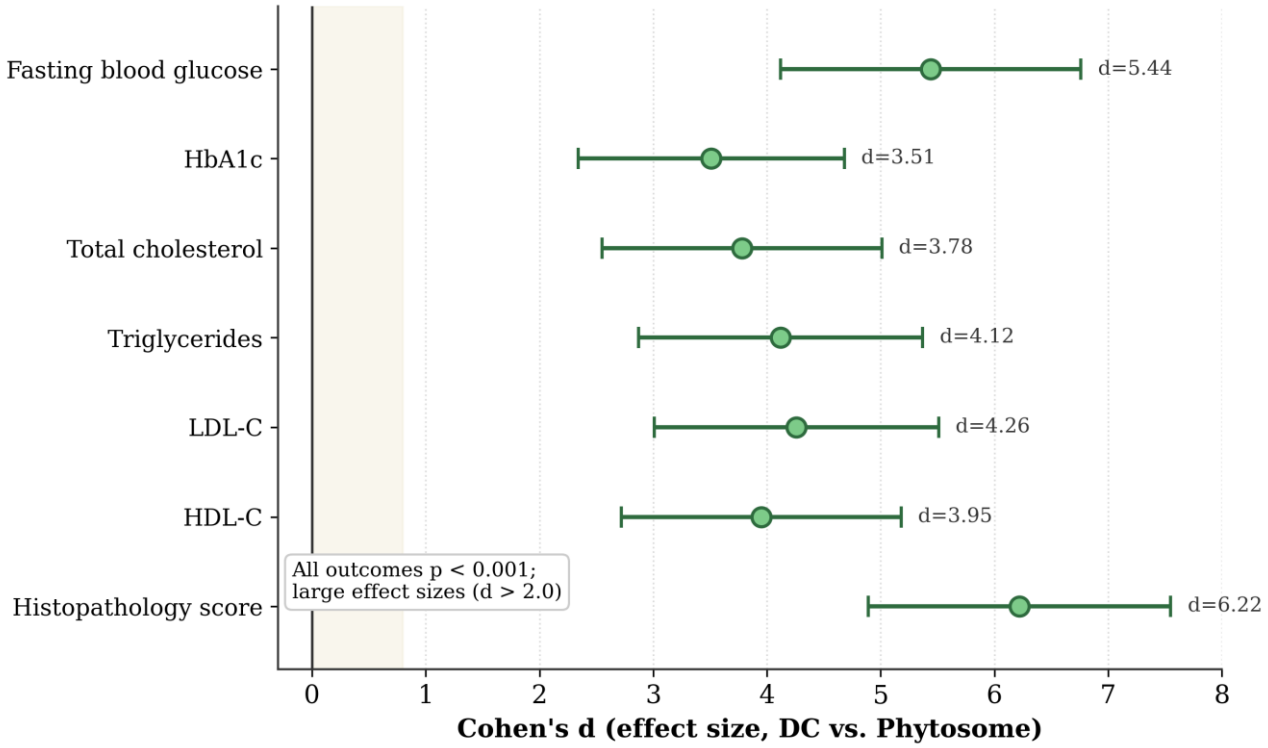


Figure 3. Forest plot of Cohen's d effect sizes (diabetic control versus *T. crispa* phytosome) across all metabolic and histopathological endpoints. Horizontal bars denote 95% confidence intervals; all effect sizes are large (d > 2.0).

4. Discussion

4.1. Validity of the diabetes model and randomization

These findings confirm the validity of the STZ model and adequate randomization. The 55 mg/kg dose produced consistent severe hyperglycemia, consistent with the established protocol for type-1-like diabetes induction.[14] The 100% induction rate compares favorably with the 80–95% typically reported, likely reflecting freshly prepared STZ in ice-cold citrate buffer administered immediately after dissolution.[14]

4.2. Mechanism of the pharmacokinetic enhancement

The 3.14-fold enhancement is consistent with the two- to five-fold improvements reported for other phospholipid-complexed phytoconstituents, including polyphenol-enriched fractions,[10] curcumin,[11] and silybin.[12] Mechanistically, molecular complexation of berberine with phosphatidylcholine creates an amphiphilic unit cell with an improved membrane-partitioning coefficient, facilitating transcellular absorption;[9] the phospholipid coating protects berberine from gastrointestinal degradation; the complex undergoes preferential intestinal lymphatic absorption via chylomicron incorporation, partially bypassing hepatic first-pass metabolism;[15] and the negative zeta potential (−32.4 mV) confers colloidal stability and may reduce P-glycoprotein-mediated efflux.[8] The shortened T_{max} supports faster dissolution, while the prolonged $t_{1/2}$ and MRT indicate sustained exposure.

4.3. Glycemic control and dual-pathway targeting

The superior glycemic control of the phytosome reflects enhanced bioavailability translating into higher sustained plasma berberine at target tissues. Berberine's anti-hyperglycemic action is primarily mediated by AMPK activation,[5] and a systematic review and meta-analysis of randomized controlled trials confirmed that berberine improves fasting glucose, HbA_{1c}, and insulin resistance in T2DM patients.[16] The greater improvements here likely reflect phytosome-enhanced bioavailability plus multi-constituent synergy, in which diterpenoids such as borapetoside C complement berberine through IRS-1/PI3K/Akt-mediated insulin sensitization — distinct from berberine's predominantly insulin-independent AMPK pathway.[6,7] This dual-pathway targeting explains the near-metformin-equivalent efficacy.

4.4. Mechanism of the lipid-lowering effect

The hypolipidemic activity is mechanistically linked to AMPK-mediated regulation: activated AMPK inactivates sterol regulatory element-binding protein (SREBP), reducing de novo lipogenesis, while berberine-type alkaloids lower circulating lipids by activating the AMPK/SREBP2 axis and downregulating proprotein convertase subtilisin/kexin type 9 (PCSK9), thereby upregulating hepatic LDL-receptor expression — analogous to statin therapy.[17] This profile is clinically relevant because atherogenic diabetic dyslipidemia is a major contributor to cardiovascular morbidity and mortality in T2DM.[18]

4.5. Beta-cell protection

Streptozotocin is a beta-cell-specific toxicant causing injury through DNA alkylation, oxidative stress, and mitochondrial dysfunction,[19] and the phytosome's protection is likely mediated by antioxidant scavenging of reactive oxygen species, anti-inflammatory NF-κB suppression by berberine, and enhancement of beta-cell survival signaling by diterpenoid constituents. Comparable beta-cell-protective and glucose-lowering effects of standardized plant extracts have been documented in STZ models,[20] and the restored fasting-insulin levels corroborate the histopathological evidence of preserved beta-cell mass.

4.6. Magnitude of effect and translational relevance

These large effect sizes reflect the controlled conditions of the animal model and would likely translate to more moderate but clinically meaningful effects in humans. Nevertheless, the comparable efficacy of the phytosome to metformin across glycemic, lipid, and histopathological endpoints supports its potential as a complementary herbal therapeutic.

4.7. Clinical implications and Indonesian herbal-medicine context

These findings carry implications for evidence-based development of traditional Indonesian herbal medicines. *T. crispa* has been used in *jamu* for diabetes for centuries, yet clinical adoption has been limited by inconsistent preparation, variable potency, and bioavailability constraints; standardized *jamu* anti-diabetic formulations have only recently been characterized mechanistically for glucose-uptake and insulin-secretory activity.[21] The phytosome platform provides a standardized, reproducible approach bridging traditional knowledge with modern pharmaceutical science, aligned with the broader convergence of phytochemistry and nanotechnology now explored for T2DM.[22] The near-equivalent efficacy to metformin supports potential clinical application and positions this study as a preclinical foundation for registration under Indonesia's BPOM *fitofarmaka* pathway.

4.8. Dose rationale and traditional-medicine correlation

The 400 mg/kg dose was selected by allometric scaling from traditional *jamu* dosing and prior preclinical studies. Traditionally, *brotowali* stem is consumed at 15–30 g fresh stem per day (≈3–6 g dried), yielding ≈75–150 mg berberine equivalent based on the 2.47% berberine content. Using the FDA body-surface-area factor (rat dose = human dose × 6.2), 400 mg/kg in rats corresponds to ≈64.5 mg/kg in humans, or 3.9–4.5 g of extract per day for a 60–70 kg adult, containing ≈96–111 mg berberine — consistent with the traditional range and well within the 500–1500 mg/day berberine doses shown safe and efficacious in human trials.[13,16]

4.9. HOMA-IR considerations and model limitations

The non-significant HOMA-IR result ($p = 0.214$; Table 3) warrants discussion, as it appears paradoxical given improvements in both fasting glucose and insulin. In the STZ model, the primary pathology is beta-cell destruction rather than peripheral insulin resistance;[19] consequently HOMA-IR may not accurately reflect insulin sensitivity, because (a) markedly reduced insulin in DC rats yields artefactually low HOMA-IR despite severe derangement, and (b) partial insulin restoration in treatment groups raises the numerator, offsetting the glucose-reduction effect. Future studies using the high-fat-diet plus low-dose STZ model, which better recapitulates the insulin-resistance-

predominant pathophysiology of human T2DM,[14] would provide more meaningful HOMA-IR data.

4.10. Strengths and limitations

Strengths include the dual pharmacokinetic–pharmacodynamic design linking enhanced bioavailability to improved outcomes, comprehensive metabolic endpoints, and metformin as an active comparator. Limitations: the STZ model primarily represents type-1-like beta-cell destruction and may not fully recapitulate human T2DM; the 28-day duration captures medium-term efficacy but not long-term safety or organ-specific toxicity; and oral gavage ensures precise dosing but does not replicate voluntary consumption, so palatability and compliance were not assessed.

5. Conclusion

A phytosome formulation of *T. crispa* stem extract achieves a 3.14-fold enhancement in the oral bioavailability of berberine (AUC_{0-24}: 1524.7 ± 185.4 versus 486.3 ± 62.8 ng·h/mL, $p < 0.001$). It also improves anti-diabetic efficacy in STZ-induced diabetic rats compared with the free extract. Glycemic control, lipid normalization, and pancreatic beta-cell protection were comparable to metformin over 28 days. The phytosome group showed a 54.8% reduction in fasting blood glucose (148.6 ± 19.2 versus 328.4 ± 42.5 mg/dL, $p < 0.001$, Cohen's $d = 5.44$), a 33.7% reduction in HbA_{1c} (6.1 ± 0.6 versus 9.2 ± 1.1%, $p < 0.001$), and significant improvements across all lipid parameters, with the mechanism attributed to enhanced AMPK activation by berberine and IRS-1/PI3K/Akt sensitization by diterpenoid constituents at therapeutically relevant plasma concentrations achieved through phospholipid complexation. These findings provide preclinical evidence supporting the development of *T. crispa* phytosome as a standardized herbal complementary therapy for diabetes mellitus, warranting progression to chronic-toxicity studies, dose-optimization trials, and ultimately Phase I/II clinical trials within the Indonesian *fitofarmaka* framework.

Declarations

Use of Generative Artificial Intelligence. The authors declare that no generative artificial intelligence tool was used in the conception, design, conduct, analysis, or interpretation of this study, nor to generate any data, figure, or reference. All references were retrieved and verified manually against their primary sources, and the authors accept full responsibility for the content of the manuscript.

Author Contributions. D.S. conceived and designed the study, supervised the pharmacokinetic and efficacy experiments, performed the statistical analysis, and drafted the manuscript. T.I.J. contributed to the study design, phytosome characterization, and interpretation of the imaging and histopathological data. B.H. supervised the laboratory analyses, validated the LC-MS/MS and ELISA procedures, and critically revised the manuscript. All authors reviewed, revised, and approved the final version and agree to be accountable for all aspects of the work.

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Conflict of Interest. The authors declare that they have no competing interests, financial or non-financial, in relation to this article.

Ethical Approval. All procedures involving animals were approved by the CMHC Ethics Committee, Indonesia (Approval No. 2024/0198) and were conducted in accordance with the Declaration of Helsinki, the National Guidelines for Health Research Ethics of the Republic of Indonesia, and institutional and international guidelines for the care and use of laboratory animals. Reporting follows the ARRIVE 2.0 guidelines.

Data Availability. The per-animal pharmacokinetic and efficacy datasets supporting the findings of this study are available from the corresponding author on reasonable request.

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