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Evaluation of the Antidiabetic and Antioxidant activities of *Ochthochloa compressa* in STZ-induced diabetic rats

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ABSTRACT

Ochthochloa compressa (Forssk.) Hilu is a medicinal grass of the Poaceae family reported to possess antidiabetic and antioxidant phytoconstituents, but its poor aqueous solubility limits the oral bioavailability of the unformulated extract. This study aimed to formulate a phospholipid-complexed phytosome of *O. compressa* extract and to evaluate its antidiabetic and antioxidant activity in streptozotocin (STZ)-induced diabetic rats. The phytosome was prepared by the thin-film hydration method using soy phosphatidylcholine and optimised across four extract-to-phospholipid ratios. The optimised formulation (1:3) showed a particle size of 217.2 nm, a polydispersity index of 0.324, a zeta potential of −28.6 mV, and an entrapment efficiency of 89.5%. FTIR confirmed extract–phospholipid interaction, and SEM revealed a nanostructured particulate morphology. In vitro release was sustained, reaching 76.4% at 8 hours. Diabetes was induced in Wistar rats by a single intraperitoneal dose of STZ (60 mg/kg); animals were then treated orally for 15 days with glibenclamide (0.5 mg/kg) or the phytosome (100 or 300 mg/kg). The phytosome produced a dose-dependent reduction in fasting blood glucose, improved body weight and lipid profile, lowered elevated liver and kidney function markers, and restored SOD, catalase, GSH and MDA levels toward normal, with the 300 mg/kg dose approaching the efficacy of glibenclamide. Pancreatic histopathology showed preserved islet architecture in phytosome-treated groups relative to the diabetic control. These findings indicate that phytosome complexation is a promising strategy for improving the antidiabetic and antioxidant performance of *O. compressa* extract.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder of carbohydrate, protein and lipid metabolism characterised by persistent hyperglycaemia arising from defects in insulin secretion, insulin action, or both [1]. The global burden of diabetes continues to rise sharply: the International Diabetes Federation estimated a global prevalence of 11.1% in 2024, projected to reach 12.96% by 2050 [2], while India's ICMR–INDIAB study reported a national diabetes prevalence of 11.4% alongside 15.3% prediabetes [3]. Chronic hyperglycaemia drives oxidative stress through increased generation of reactive oxygen species and impaired antioxidant defences, which in turn contributes to the vascular, hepatic and renal complications of diabetes [4,5].

Long-term pharmacotherapy with synthetic antidiabetic agents is frequently limited by adverse effects and secondary treatment failure, which has renewed interest in plant-derived antidiabetic and antioxidant agents [6]. *Ochthochloa compressa* (Forssk.) Hilu, a medicinal grass of the Poaceae family, has been reported to contain alkaloids, flavonoids, tannins and phenolic compounds with antidiabetic, antioxidant and antimicrobial activity [7–9]. However, like many polyphenol-rich plant extracts, its therapeutic potential is constrained by poor lipid solubility and consequently low oral bioavailability. Phytosome technology addresses this limitation by complexing plant constituents with phospholipids such as soy phosphatidylcholine, yielding a lipid-compatible nanostructure with improved membrane permeability and bioavailability relative to the unformulated extract [10,11].

The present study was therefore undertaken to formulate a phytosome complex of *O. compressa* extract, to characterise it for particle size, zeta potential, entrapment efficiency, surface morphology and drug release, and to evaluate its antidiabetic and antioxidant efficacy in streptozotocin (STZ)-induced diabetic rats in comparison with the standard antidiabetic drug glibenclamide.

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Materials and Methods

Plant material, chemicals and equipment

A standardised *O. compressa* extract (50:1, brown fine powder) was procured from a certified commercial supplier with an accompanying certificate of analysis and was independently re-verified by qualitative phytochemical testing on receipt. Soy phosphatidylcholine, streptozotocin, and analytical-grade solvents (methanol, dichloromethane) were obtained from Sigma-Aldrich/Merck. Glibenclamide served as the standard reference antidiabetic drug. Characterisation was performed using a dynamic/electrophoretic light-scattering particle size analyser (Malvern Panalytical), an FTIR spectrophotometer, and a scanning electron microscope (Zeiss).

Preliminary phytochemical screening

The extract was screened qualitatively for alkaloids (Mayer's test), flavonoids (Shinoda test), tannins and phenolic compounds (ferric chloride test), saponins and terpenoids using standard pharmacognostic procedures [12].

Preparation of the O. compressa phytosome

The phytosome was prepared by the thin-film hydration method [13].

The extract and soy phosphatidylcholine were dissolved together in methanol–dichloromethane and subjected to rotary evaporation under reduced pressure to deposit a thin film, which was further dried under vacuum.

The film was hydrated with phosphate buffer (pH 7.4) under gentle rotation to form a phytosome suspension, which was then probe-sonicated to reduce and homogenise vesicle size (Figure 1).

Four extract-to-phospholipid ratios (1:0.5, 1:1, 1:2 and 1:3) were prepared and compared; the 1:3 ratio (100 mg extract : 300 mg phosphatidylcholine) gave the smallest particle size and highest entrapment efficiency and was selected as the optimised formulation for all subsequent studies.

Characterisation of the phytosome

Particle size, polydispersity index (PDI) and zeta potential of the optimised formulation were determined by dynamic and electrophoretic light scattering. Entrapment efficiency (EE%) was calculated from the free (unentrapped) extract remaining after separation, expressed as a percentage of the total extract used. Surface morphology was examined by SEM at 20,000× magnification. FTIR spectra (4000–400 cm^{-1}) of the free extract and the phytosome were compared to identify shifts in characteristic O–H, C–H, C=O/C=C and C–O bands indicative of extract–phospholipid interaction.

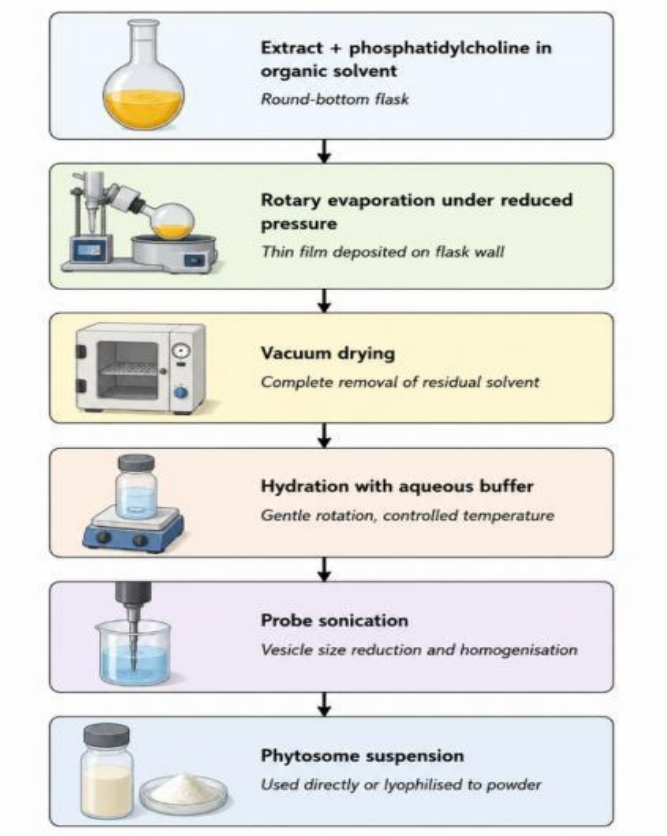

Figure 1. Schematic of Ochthochloa compressa phytosome preparation by the thin-film hydration method.

In-vitro drug release study

Cumulative drug release from the optimised phytosome was determined over 8 hours using a gallic-acid-equivalent UV-spectrophotometric assay (λmax 276 nm), referenced against a gallic acid calibration curve ($y = 0.0468x + 0.0128$, $R^2 = 0.9969$) constructed over 0–10 µg/mL.

Experimental animals and induction of diabetes

Healthy adult Wistar albino rats (180–250 g, 8–12 weeks, both sexes) were acclimatised for 15 days under standard husbandry conditions (25 ± 2°C, 50 ± 5% RH, 12 h light/dark cycle) with free access to feed and water. The study was approved by the Institutional Animal Ethics Committee (IAEC/CPCSEA) of K.M. College of Pharmacy, Madurai, and conducted in accordance with CPCSEA guidelines [14] and the 3Rs principle. Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg) freshly dissolved in citrate buffer (0.1 M, pH 4.5) [15,16]; successful induction was confirmed 48–72 hours later by a fasting blood glucose ≥250 mg/dL.

Experimental design and grouping

Confirmed diabetic animals were randomly allocated to five groups (n = 5 per group) and dosed orally, once daily, for 15 days:

Table 1. Experimental grouping and treatment schedule (15-day oral dosing)

Group	Treatment
I – Normal control	No diabetes induction; vehicle only
II – Diabetic control	STZ (60 mg/kg, i.p.), no treatment
III – Standard	STZ + glibenclamide 0.5 mg/kg p.o.
IV – Low-dose phytosome	STZ + *O. compressa* phytosome 100 mg/kg p.o.
V – High-dose phytosome	STZ + *O. compressa* phytosome 300 mg/kg p.o.

Biochemical, antioxidant and histopathological evaluation

Body weight and fasting blood glucose were recorded at baseline and at intervals throughout treatment. On day 15, terminal blood samples were collected for lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C) and liver/kidney function markers (AST, ALT, urea, creatinine). Pancreatic tissue homogenates were assayed for superoxide dismutase (SOD) [17], catalase [18], reduced glutathione (GSH) [19] and malondialdehyde (MDA) [20]. Pancreatic sections were fixed in 10% neutral buffered formalin, processed, and stained with haematoxylin and eosin for histopathological examination of islet architecture.

Statistical analysis

Data are expressed as mean ± SEM. Group comparisons were performed by one-way ANOVA followed by Dunnett's post-hoc test using GraphPad Prism/SPSS, with $p < 0.05$ considered statistically significant.

Results and Discussion

Phytochemical screening and formulation optimisation

Preliminary screening confirmed the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins and terpenoids in the *O. compressa* extract; steroids were not detected. Among the four extract-to-phospholipid ratios evaluated, increasing the phospholipid proportion progressively reduced particle size and PDI while improving entrapment efficiency (Table 2). The 1:3 ratio (F4) gave the smallest particle size (218.7 nm), lowest PDI (0.214) and highest entrapment efficiency (89.5%), and was selected as the optimised formulation.

Table 2. Effect of extract-to-phosphatidylcholine (PC) ratio on phytosome characteristics.

Batch	Extract : PC ratio	Particle size (nm)	PDI	EE (%)
F1	1 : 0.5	612.4	0.482	58.6
F2	1 : 1	487.9	0.401	67.3
F3	1 : 2	329.5	0.312	76.9
F4 (optimised)	1 : 3	218.7	0.214	89.5

Particle size, zeta potential, surface morphology and FTIR

Dynamic light scattering of the optimised (re-measured) formulation confirmed a mean particle size of 217.2 nm with a PDI of 0.324, and a zeta potential of −28.6 mV, indicating a satisfactorily homogeneous, nanosized and electrostatically stable vesicle population (Figure 2). SEM revealed irregular-to-spherical, closely packed submicron particles with a rough surface and some agglomeration (Figure 3). FTIR spectra of the free extract and the phytosome both showed O–H (~3300–3400 cm^{-1}), C–H (~2920–2950 cm^{-1}), C=O/C=C (~1600–1650 cm^{-1}) and C–O (~1000–1200 cm^{-1}) bands; retention of these bands with shifts in intensity and position in the phytosome spectrum supported extract–phospholipid complexation (Figure 4).

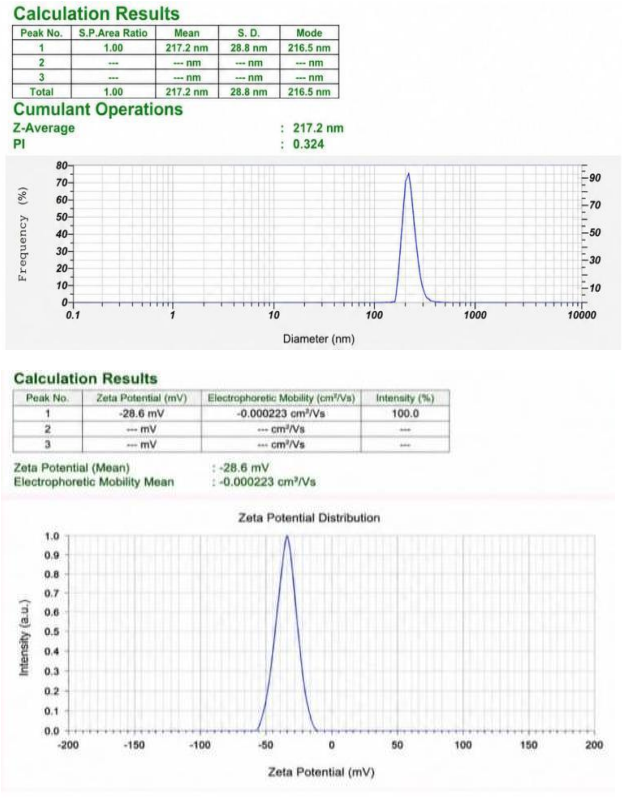

Figure 2. Particle size (top) and zeta potential (bottom) distribution of the optimised phytosome.

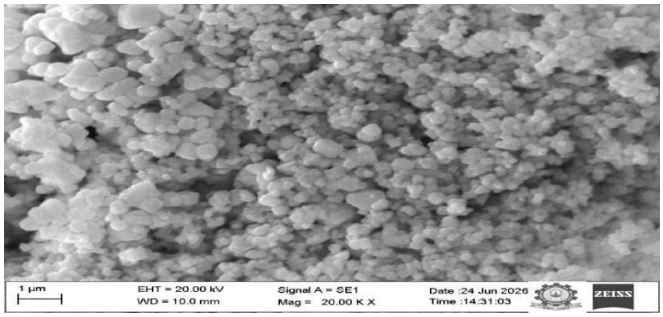

Figure 3. SEM photomicrograph of the optimised phytosome (20,000×, 1 µm scale bar).

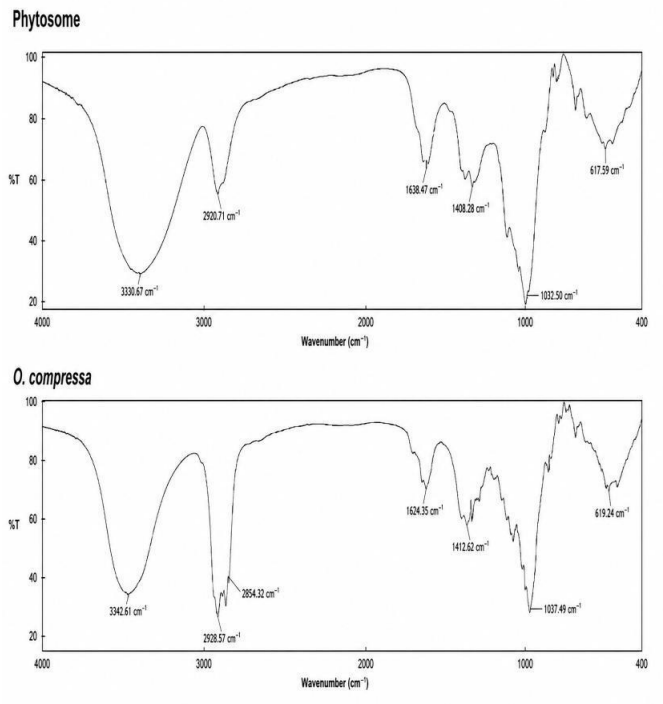

Figure 4. FTIR spectra of the phytosome (top) and free O. compressa extract (bottom).

In-vitro drug release

The phytosome exhibited a gradual, sustained release profile, with cumulative release of 18.5%, 31.2%, 48.7%, 63.8% and 76.4% at 1, 2, 4, 6 and 8 hours, respectively (Figure 5).

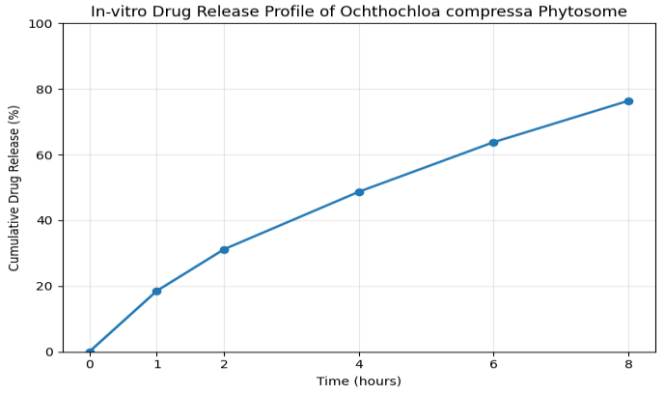

Figure 5. In vitro cumulative drug release profile of the optimised phytosome (0–8 h).

Effect on fasting blood glucose and body weight

STZ administration produced a marked rise in fasting blood glucose (FBG) and a decline in body weight in the diabetic control group relative to normal controls. Treatment with the phytosome produced a dose-dependent reduction in FBG over the 15-day period: by day 15, FBG had fallen by 50.8% in the glibenclamide group, 45.9% in the 300 mg/kg phytosome group and 33.8% in the 100 mg/kg phytosome group, relative to each group's day-3 peak ($p < 0.05$–0.01 vs. diabetic control) (Figure 6). Body weight showed a corresponding pattern: the diabetic control group lost 7.0% body weight over the study period, whereas the 300 mg/kg phytosome group gained 8.0% and the glibenclamide group gained 20.4% (Figure 7).

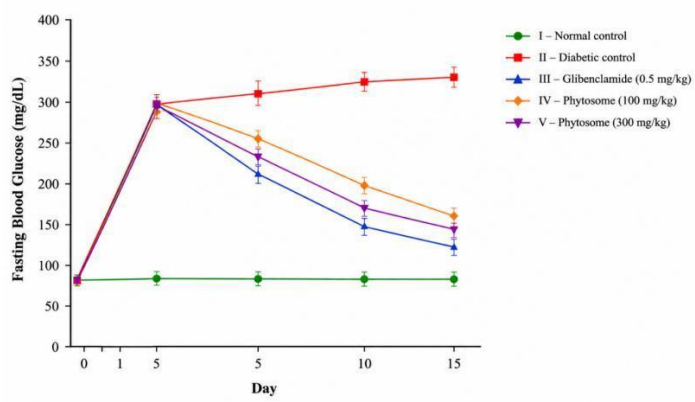

Figure 6. Effect of O. compressa phytosome on fasting blood glucose over 15 days.

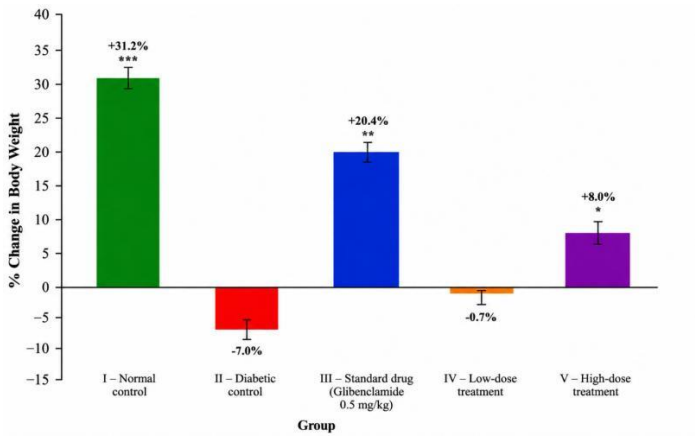

Figure 7. Percentage change in body weight across experimental groups.

Effect on lipid profile

The diabetic control group showed marked dyslipidaemia (elevated total cholesterol, triglycerides and LDL-C, and reduced HDL-C) relative to normal controls. Treatment with glibenclamide or the phytosome reduced total cholesterol, triglycerides and LDL-C and improved HDL-C, with the 300 mg/kg phytosome dose outperforming the 100 mg/kg dose (Figure 8).

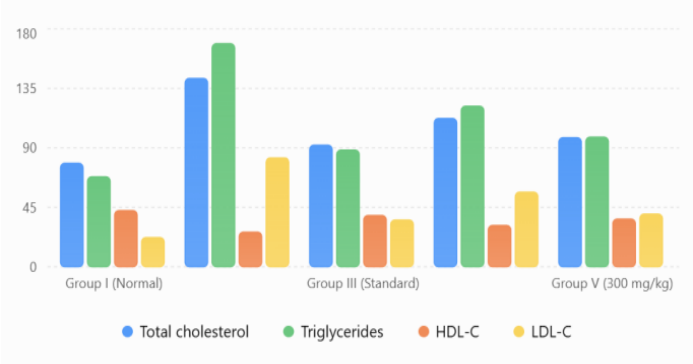

Figure 8. Effect of O. compressa phytosome on serum lipid profile on day 15.

Effect on liver and kidney function markers

AST, ALT, urea and creatinine were all significantly elevated in diabetic control animals, indicating hepatic and renal stress. All treatment groups showed reductions toward normal values, with the greatest improvement in the glibenclamide and 300 mg/kg phytosome groups (Figure 9).

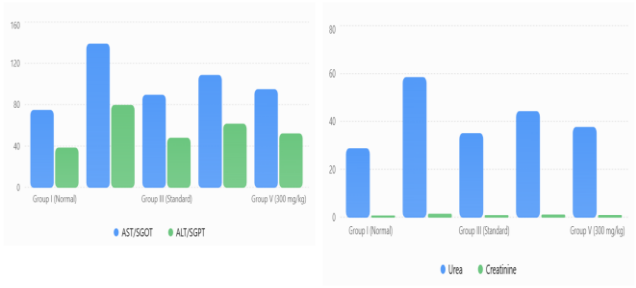

Figure 9. Effect on liver function markers, AST/ALT (left) and kidney function markers, urea/creatinine (right).

Effect on antioxidant parameters

SOD, catalase and GSH were markedly decreased, and MDA markedly increased, in pancreatic tissue of diabetic control animals, indicating enhanced oxidative stress. Treatment with the phytosome restored SOD, catalase and GSH and reduced MDA toward normal values in a dose-dependent manner, with the 300 mg/kg dose producing a greater antioxidant effect than the 100 mg/kg dose (Figure 10).

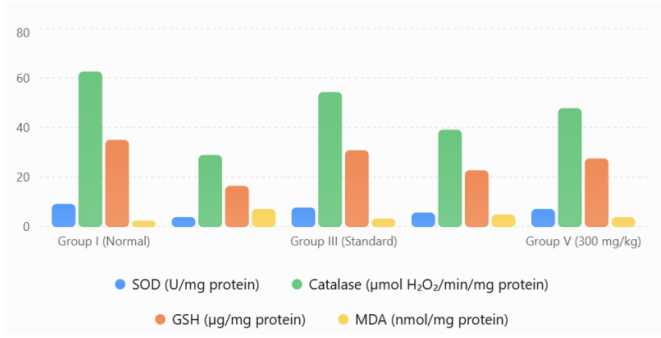

Figure 10. Effect of O. compressa phytosome on tissue antioxidant parameters (SOD, catalase, GSH, MDA).

Histopathological findings

Pancreatic sections from the diabetic control group showed reduced islet cellularity and disrupted islet architecture relative to normal controls. Sections from the glibenclamide and phytosome-treated groups showed better-preserved islet architecture, with the 300 mg/kg phytosome group showing the greatest preservation among the treated groups, consistent with the biochemical antidiabetic and antioxidant findings (Figure 11).

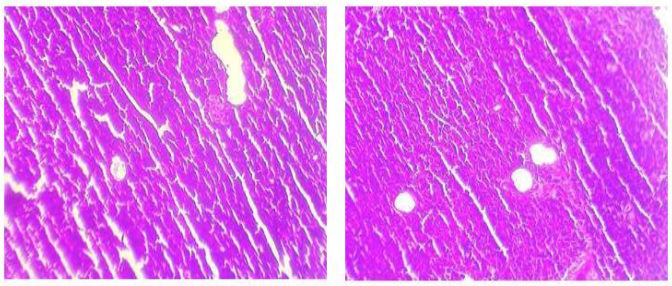

Figure 11. Representative photomicrographs of H&E-stained pancreatic sections (light microscopy).

Discussion

This study demonstrates that phospholipid complexation is an effective strategy for improving the biopharmaceutical and pharmacological performance of *O. compressa* extract. The qualitative phytochemical profile — alkaloids, flavonoids, tannins, phenolic compounds, saponins and terpenoids — is consistent with previously reported Poaceae-family medicinal grasses [7,8], and these constituents are plausible contributors to the observed antidiabetic and antioxidant effects. The FTIR data support successful phytosome formation through interaction between extract phytoconstituents and phosphatidylcholine [10], while the nanosized particles (217.2 nm), satisfactory PDI, negative zeta potential and high entrapment efficiency are comparable to benchmarks reported for other plant-derived phytosome formulations [11,21].

STZ administration produced the expected diabetic phenotype — hyperglycaemia, weight loss, dyslipidaemia, hepatic and renal biochemical stress, and oxidative imbalance — against which the phytosome produced a consistent, dose-dependent protective effect that in several parameters approached that of glibenclamide at the 300 mg/kg dose. This pattern parallels the dose-dependent antidiabetic and antioxidant responses reported for other STZ-model plant extracts and phytosome formulations [22–27], and the hepatoprotective and nephroprotective findings are consistent with reports for other antidiabetic plant extracts evaluated in the same model [28,29]. The preservation of pancreatic islet architecture in phytosome-treated groups further supports a beta-cell-

protective, rather than purely peripheral, mechanism of action, in line with histopathology-antioxidant correlations reported for comparable plant-extract studies [23].

Overall, these findings support the therapeutic promise of *O. compressa* phytosome as a herbal nanoformulation for managing diabetes-associated metabolic and oxidative disturbances, and add to the broader evidence that phytosome complexation can enhance the pharmacological performance of poorly bioavailable plant phytoconstituents relative to the unformulated extract [30,31]. Limitations of the present study include the relatively small group size and the absence of long-term toxicity and pharmacokinetic data, which should be addressed in future work.

Conclusion

An optimised *Ochthochloa compressa* phytosome was successfully formulated by the thin-film hydration method, yielding a nanosized, negatively charged, high-entrapment-efficiency formulation with sustained in vitro release. In STZ-induced diabetic rats, the phytosome produced dose-dependent improvements in fasting blood glucose, body weight, lipid profile, liver and kidney function markers, tissue antioxidant status, and pancreatic islet architecture, with the 300 mg/kg dose showing efficacy approaching that of glibenclamide. These results support *O. compressa* phytosome as a promising herbal nanoformulation warranting further pharmacokinetic and toxicological evaluation for the management of diabetes and its associated oxidative complications.

Acknowledgements

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