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Research

Antidiabetic Efficacy of Diosmetin-Loaded Phytosomes in STZ-Induced Diabetic Rats

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Abstract:
Diabetes mellitus is a long-standing metabolic disorder characterized by hyperglycemia and disturbed carbohydrate, lipid, and protein metabolism due to impaired insulin secretion or peripheral insulin resistance. A flavonoid, diosmetin, possesses excellent biological activities but is limited in clinical use because it has poor aqueous solubility, low permeability, and significant first-pass metabolism, which leads to low oral bioavailability. To overcome these limitations, a phytosomatically formulated diosmetin was designed to maximize its bioavailability and antidiabetic effects. The current research involves the successful loading of diosmetin (DS) into a phytosomal entity by the formation of bond between the soya phosphatidylcholine and diosmetin in various molar ratios. Successfully prepared DS phytosomes through complexation with phospholipids and they were characterized by physicochemical, chromatography, spectroscopy, differential scanning calorimetry (DSC), and nuclear magnetic resonance (NMR) studies. *In vivo* studies using streptozotocin which induced diabetes in albino rats, and compared the results against pure DS alone. The antidiabetic potential of DS phytosomes evaluated by an *in vivo* study revealed a significant decrease in the levels of blood glucose in the DS phytosomes-treated group as compared to plain DS. The above-mentioned results showed that the antidiabetic potency of DS was enhanced in phytosomes as compared to DS.

This research points to phytosomal encapsulation as a powerful approach to enhance the solubility, bioavailability and therapeutic interest of diosmetin and presents a worthy method for the improvement in pharmacokinetics in other poorly soluble flavonoids.

Keywords: Diosmetin, Phytosome, Bioavailability, Antidiabetic, Phospholipid Complex

1. INTRODUCTION

Diabetes mellitus is a metabolic disease involving chronic disordered carbohydrate, lipid, and protein metabolism. It is defined by chronic hyperglycaemia, glycosuria, hyperlipidaemia, and negative balance of nitrogen, and in certain instances, ketonemia [1].

Diabetes is a metabolic state mainly defined by long-term hyperglycaemia, which contributes significantly to the risk of microvascular complications like retinopathy, nephropathy, and neuropathy. It is also linked with decreased life expectancy, significant morbidity resulting from this microvascular disease, like ischemic heart disease,

stroke, and peripheral vascular disease. These together lead to a compromised quality of life in patients with diabetes [2].

According to the World Health Organization, about 346 million people are projected to have diabetes by the year 2025, and 10 % increase is expected to raise this number to around 439 million by 2030 [3].

Over the last few years, herbal medicines have become quite popular for more treatment of diabetes because of their perceived safety, lower toxicity, and fewer side effects than synthetic medicines.

Diosmetin, a plant-derived flavonoid, is commonly found in different medicinal plants and has been used traditionally to treat various diseases like asthma, gout, memory loss, polycystic ovarian disease (PCOD), cardiovascular illness, gastric ulcers, diabetes, tuberculosis, and piles. It is highly prevalent in Citrus fruits, Candle bush (*Cassia alata*), Timollanut (*Teucrium gnaphalodes*), and Garden mum (*Chrysanthemum morifolium*) [4].

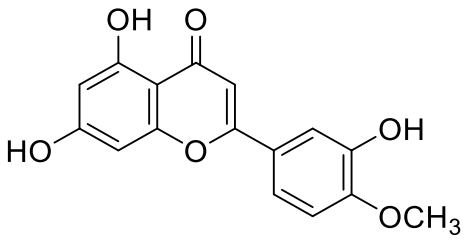

Figure 1: Diosmetin

Phytosomes are a complex of phospholipids and natural active phytochemicals, bound in their structures, obtained by the reaction between phosphatidylcholine and plant extracts in an aprotic solvent. “Phyto” means plant, and "some” suggests a cell-like entity. Phytosomes are a new drug delivery system where the active phytoconstituents are encapsulated and chemically attached to phosphatidylcholine, a key ingredient of the human cell membrane. It is an unusual structure that is cell-like and enhances better interaction with biological membranes. The encapsulation of poorly soluble polyphenolic substances as phytosomes increases their absorption dramatically, enhancing better membrane permeability and bioavailability.

This system of delivery holds great promise for surmounting solubility and permeability restrictions normally found in natural compounds [5].

Phytosomes are typically formulated by combining the active biological phytoconstituent with phospholipids such as phosphatidylcholine (PC), phosphatidylserine (PS), or phosphatidylethanolamine (PE) in specific stoichiometric proportions under stress-free conditions [6].

To date, no studies have reported on the impact of diosmetin and its phytosomal preparation on streptozotocin-induced diabetes. Inspired by this, the current research aimed to effectively load diosmetin into a phytosomal system by bonding with soya phosphatidylcholine in different molar ratios. The formation of drug-phospholipid complex was confirmed by proton nuclear magnetic resonance spectroscopy.

Additionally, the solubility profile and melting point of the synthesized complex were evaluated. The physical nature of the phytosomes was characterized using differential scanning calorimetry (DSC), while vesicle size and shape were examined with transmission electron microscopy (TEM). A comparative in vivo pharmacokinetic study of free diosmetin and its phytosomal delivery system was conducted in Wistar albino rats. Furthermore, extensive histopathological examinations of pancreatic and liver tissues were performed to investigate potential therapeutic effects facilitated by the phytoconstituents.

2. MATERIALS AND METHODS

2.1 Preparation of DS-SPC complex

An equimolar amount of diosmetin (DS) and soya phosphatidylcholine (SPC) was dissolved in 20 mL of dichloromethane (DCM) in a round-bottom flask. The mixture was stirred at room temperature with a magnetic stirrer for 2 hours, and the solvent was then evaporated. The obtained DS-SPC complex was extensively washed with n-hexane to remove free SPC and dried under vacuum later. The dried DS-SPC complex was preserved in an amber glass vial to prevent it from the light.

For the preparation of the phytosomes, cholesterol and DS–SPC complex were dissolved in DCM together, and a thin film was generated using a rotary evaporator. After leaving the thin film to dry for 24 hours, it was hydrated with phosphate-buffered saline (pH 7.4) at room temperature to produce vesicular or micellar structures, which are termed "phytosomes." [7].

2.2 Characterization of the DS-SPC complex

2.2.1 Solubility study

Solubility profiles of diosmetin (DS) and DS–SPC were compared by separately dissolving 1 mg

of each compound in 10 mL of different solvents such as distilled water, chloroform, methanol, dichloromethane, dimethyl sulfoxide (DMSO), and n-hexane in small volumetric flasks [8].

2.2.2 Melting point determination

The melting points of DS, SPC, and DS–SPC complex were determined by using the melting point apparatus and were uncorrected [9].

2.2.3 Ultraviolet-Visible Spectroscopy

The DS-SPC complex formation was examined using UV-Visible spectroscopy (Thermo Scientific) in the wavelength range of 200-800 nm. Small aliquots of colloidal samples were made in methanol and their absorbance spectra were taken.

2.2.4 Thin layer chromatographic study

Thin-layer chromatography (TLC) of diosmetin (DS) and DS–SPC complex was carried out using silica gel 60 as the stationary phase. n-hexane: ethyl acetate in a ratio of 1:9 and 7:3, respectively, taken as mobile phase. DS was dissolved in methanol and spotted on the TLC plates, which were developed with the corresponding mobile phases. The plates were viewed under a UV chamber after development. The retention factor (Rf) values of DS were noted carefully and compared with those of DS-SPC complex to validate complex formation [9].

2.2.5 ^{1}H-Nuclear Magnetic Resonance (proton-NMR) spectroscopy

Proton nuclear magnetic resonance (^{1}H-NMR) spectra of diosmetin (DS), soya phosphatidylcholine (SPC), and the DS-SPC complex were obtained according to standard procedures. Chemical shifts were referenced to tetramethylsilane (TMS) as an internal standard [10].

2.2.6 Differential Scanning Calorimetry (DSC)

Physical states of diosmetin (DS) and the DS–SPC complex were determined by Differential Scanning Calorimetry (DSC). The samples were hermetically sealed in aluminium crimp cells, and the reference material was alpha alumina powder. Scanning was carried out in a nitrogen environment over the temperature range 30-300°C at a heating rate of 10°C/min. Calibration of temperature was conducted from time to time using indium as the standard [11].

2.2.7 X-Ray diffraction (XRD)

X-ray diffraction study describes an effective way to procure information on the crystallinity and shape. Powder X-ray diffraction (PXRD) patterns revealed the arrangement of molecules in the crystals. The solid-state characterizations of flavonoid and flavonoid-SPC complex were performed by X-ray diffractometer [12].

2.3 Characterization of the vesicular system

2.3.1 Vesicle shape and size

The morphology of the developed phytosomes was studied by Scanning Electron Microscopy (SEM). The nano-vesicles were seen under SEM, and microphotographs were taken at appropriate magnifications to scan their surface features.

The vesicle size and finer structural details were also studied by Transmission Electron Microscopy (TEM). The phytosomal preparations were viewed under TEM, and photographs were taken at adequate magnifications to study vesicle size and homogeneity [13].

2.3.2 Entrapment efficiency

The phytosomal dispersion was centrifuged in a cooling centrifuge (Remi) at 12,000 rpm for 1 hour at 4 °C. After centrifugation, the supernatant was gently separated to determine an estimate of the unentrapped diosmetin. The supernatant was read for its absorbance at 253 nm using a UV-Visible spectrophotometer. The sediment was lysed by adding 1 mL of 0.1% Triton X-100 and then diluted to 100 mL using phosphate-buffered saline (PH 7.4). The absorbance at 253nm was measured to calculate the quantity of diosmetin trapped inside the vesicles. The total diosmetin content in 1ml of dispersion was determined from the addition of diosmetin from the supernatant and the sediment [14].

The entrapment efficiency percentage (%EE) was determined by the following formula:

$$Percent\ entrapement = \frac{Amount\ of\ drug\ in\ sediment}{Total\ amount\ of\ drug\ added}\ x\ 100$$

2.3.4 Stability studies

The optimized phytosome batch was then analyzed for stability under three storage conditions, at room temperature, refrigeration (2-8°C), and accelerated storage (40 ± 2 °C) for 90 days. The formulation was intermittently checked throughout these 90 days for any alteration in the morphological parameters such as vesicle shape, size, and integrity to test its physical stability [15].

***2.4 In Vitro* Antioxidant Activity**

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals, leading to chain reactions that may damage the cells of organisms.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

DPPH assay is a rapid and economical method, which is commonly employed for the assessment of antioxidative capacity of diverse natural compounds. Weigh 0.001 mole (4 mg) DPPH and dissolve in 100 mL methanol to obtain a deep purple-colored solution. Take different concentrations (10ug/ml – 50ug/ml) of test material (0.001mole). Prepare a test sample in methanol. Mix a standard volume of the DPPH solution (3ml) with test material (1ml) and methanol (6ml) in 10 ml volumetric flask. Incubate it in the dark for a certain period, normally 30 minutes. Determine the absorbance of the formed solution at 517 nm using a spectrophotometer [8]. Calculate the percent of DPPH radical scavenging activity using the formula:

$$Scavenging\ Activity\ (\%) = \frac{A\ control - A\ sample}{Acontrol}\ x\ 100$$

2.5 *In vivo* studies

Animals

With the Institutional Animal Ethics Committee (IAEC) approval, the *in vivo* experiments were performed according to the Committee for Control and Supervision of Experiments on Animals (CCSEA) and OECD guidelines on Wistar albino rats (8–10 weeks old) with weights ranging from 200–250 g. The animals were kept in standard polypropylene cages (four rats per cage) under controlled environmental conditions that consisted of a temperature of 25–26 °C and a 12-hour day/night cycle. They were provided free access to clear drinking water and received a normal rodent diet. All experiments were performed between 07:00 and 16:00 hours. Before the study, the animals were acclimatized to the lab for one week [16].

2.5.1 Evaluation of antidiabetic potential

2.5.1.1 Streptozotocin-induced diabetic model

The rats were divided into five groups. The following groups were utilized for the 50-day duration protocol: Group-I (vehicle control), Group-II (streptozotocin; 32.5mg/kg p.o.), Group-III (diosmetin; 20 mg/kg in normal saline), Group-IV (diosmetin phytosomes; equivalent to 20 mg/kg of diosmetin in normal saline) and Group-V (metformin; 20 mg/kg in normal saline).

2.6 Histopathological studies

Histological examinations of the pancreas and liver tissue were conducted through selective staining with hematoxylin and eosin (H&E). Sections of the tissue, 7 to 10 µm thick, were first fixed in formal-calcium solution and paraffin wax-embedded. The sections were dewaxed using xylene and rehydrated in a decreasing gradient of ethanol concentrations. After hydration, the sections were stained with hematoxylin, dehydrated with progressively higher grades of alcohol to 70%, and stained again with alcoholic eosin solution at 1%. The sections were then differentiated in 90% ethanol, cleared in xylene, mounted gently on glass slides, and viewed under a light microscope for histopathological assessment [17].

3. RESULTS AND DISCUSSION

3.1 Solubility Studies

The solubility profile of the diosmetin–phospholipid complex (DS-SPC) was studied and compared with that of the pure diosmetin in different solvents, which include distilled water, dichloromethane, methanol, chloroform, dimethylsulphoxide (DMSO), and n-hexane. Pure diosmetin demonstrated significant solubility in non-polar as well as semi-polar solvents like dichloromethane, DMSO, chloroform, and n-hexane. However, it demonstrated poor solubility in methanol and no solubility at all in distilled water. When complexed with phospholipids, the DS-SPC complex exhibited a unique solubility pattern: Solubility in DMSO and methanol, Poor solubility in dichloromethane, chloroform, and n-hexane, and Insolubility in distilled water was unaffected. This change in solubility pattern reflects a change in the polarity compatibility of the molecule. The increased solubility in polar organic solvents like methanol and DMSO indicates better dissolution properties. This better solubility profile of the DS-SPC complex can be responsible for better bioavailability in biological surroundings, possibly overcoming the shortcomings related to the poor aqueous solubility of pure diosmetin.

3.2 Melting Point Determination

There was a clear difference in melting points between pure diosmetin (DS), soya phosphatidylcholine (SPC), and the complex of diosmetin–phospholipid (DS-SPC). Pure diosmetin had a melting point between 262–264°C, reflecting its crystalline nature. This is slightly higher than the previously reported melting point of diosmetin, which is 258°C [18]. The DS-SPC complex, however, had a lower melting point of about 258°C. This lowering of the melting point upon complexation indicates successful complex formation of the DS-SPC complex. SPC addition to the drug matrix would be expected to destabilize the

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ordered crystalline lattice of diosmetin, thereby reducing the lattice energy and subsequently the melting point. This evidence is consistent with the hypothesis that a molecular interaction between DS and SPC took place upon complex formation, resulting in an altered physicochemical profile.

3.3 Ultraviolet-Visible (UV-Vis) Spectroscopy

An ultraviolet-visible spectral analysis was conducted to study potential interactions between diosmetin and soya phosphatidylcholine (SPC) upon complex formation in the DS-SPC complex. Pure diosmetin's maximum absorption wavelength (λmax) was at 253 nm due to its conjugated system. However, the DS-SPC complex had a slightly blue-shifted λmax at 247 nm. This bathochromic shift (shorter wavelength) suggests that there is a modification of the electronic environment of the diosmetin molecule, probably due to complexation with the phospholipid. The spectral shift observed confirms the formation of the DS-SPC complex, proposing molecular interactions including hydrogen bonding or van der Waals forces between SPC and diosmetin, leading to the modification of electronic transition features of the flavonoid.

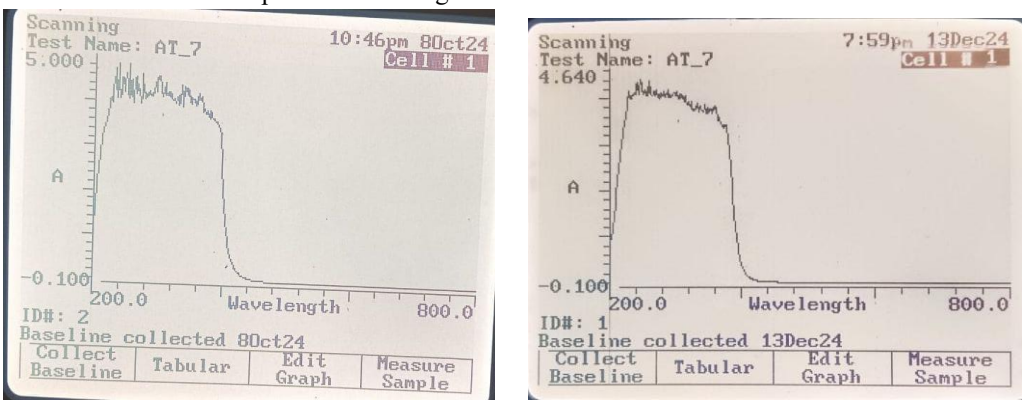

Figure 2: UV-Vis spectra A) Diosmetin B) DS-SPC complex

3.4 Thin Layer Chromatographic (TLC) Investigation

Thin layer chromatography was used to assess the physicochemical alterations upon complexation of diosmetin (DS) with soya phosphatidylcholine (SPC). The Rf value (retention factor) of pure diosmetin was established to be 0.87, whereas the DS–SPC complex had an ever so slightly higher Rf value of 0.92 under identical chromatographic conditions. This slight marginal rise in the Rf value indicates an adjustment in the polarity and mobility of the compound, which would be an outcome of the DS–SPC complex formation. The phospholipid interaction might cause a more lipophilic nature of the complex so that it can move a longer distance on the TLC plate. This change in the Rf value also indicates successful complexation between diosmetin and SPC.

3.5 Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy

^{1}H-NMR spectroscopy was utilized to determine the structural interactions between diosmetin (DS) and soya phosphatidylcholine (SPC) in the DS-SPC complex formation. The NMR spectrum of pure diosmetin showed a typical downfield signal at 12.94 ppm, which was attributed to the hydroxyl proton at the 3-position (3-OH) of the flavonoid skeleton. This sharp signal is characteristic of the occurrence of a free hydroxyl group participating in hydrogen bonding or intra/intermolecular interaction. Conversely, the ^{1}H-NMR spectrum of the DS-SPC complex indicated OH proton signal at 11.7 ppm, indicating that the hydroxyl group was involved in a chemical interaction. The spectrum of the complex also revealed new peaks due to the choline moiety of SPC, confirming successful entrainment of the phospholipid. These changes in the spectrum are indicative of the formation of a DS–SPC complex, in which the hydrogen atom from the 3-OH group would be displaced to facilitate interaction between the oxygen atom of diosmetin and the polar head group of phosphatidylcholines. This interaction strongly indicates the formation of a non-covalent or hydrogen-bonded complex, responsible for the altered physicochemical behaviour reported in other characterization studies.

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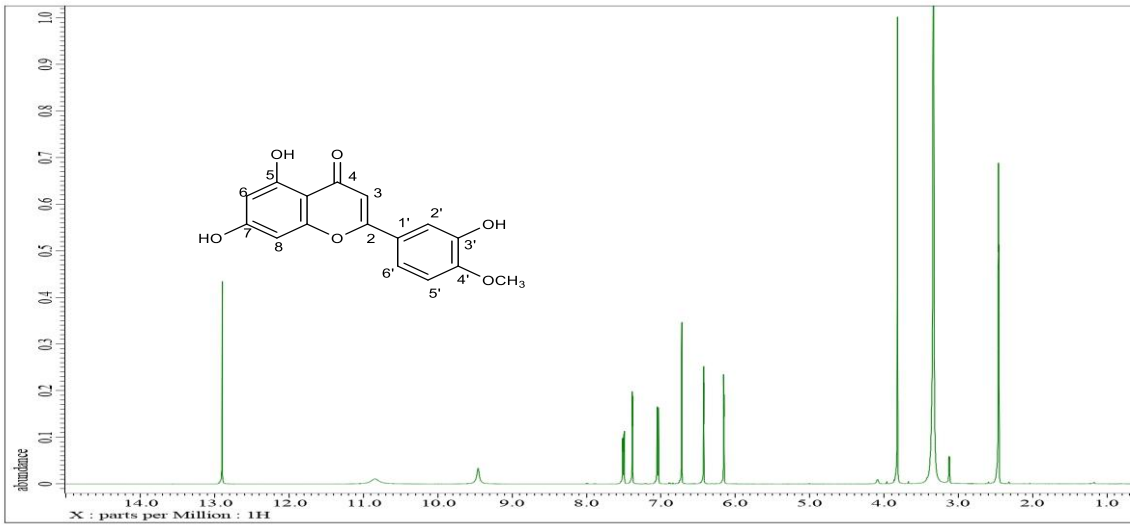
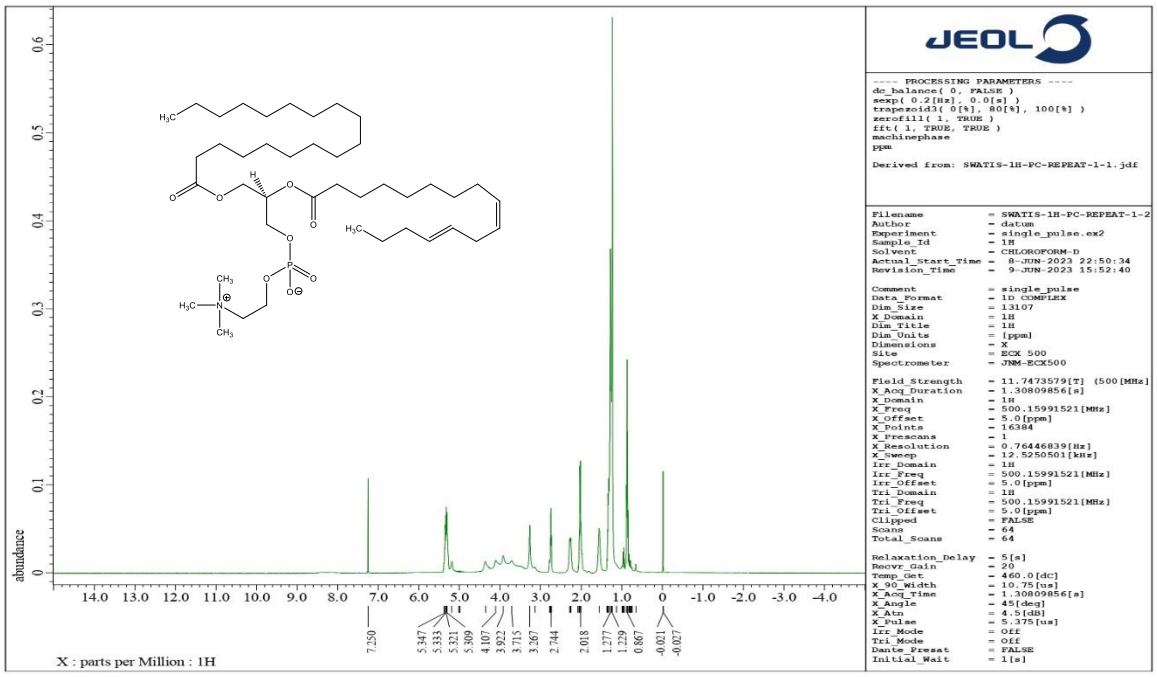
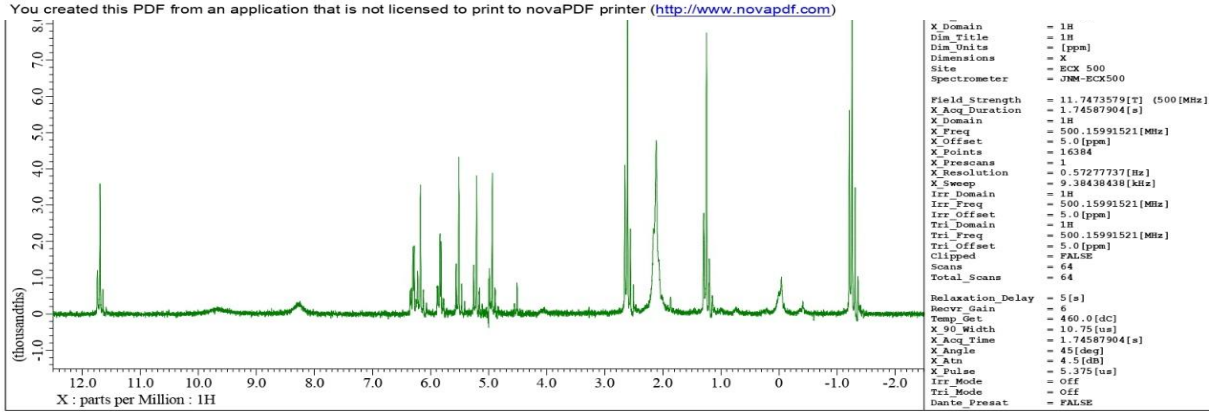


Figure 3: NMR Spectrum A) Diosmetin





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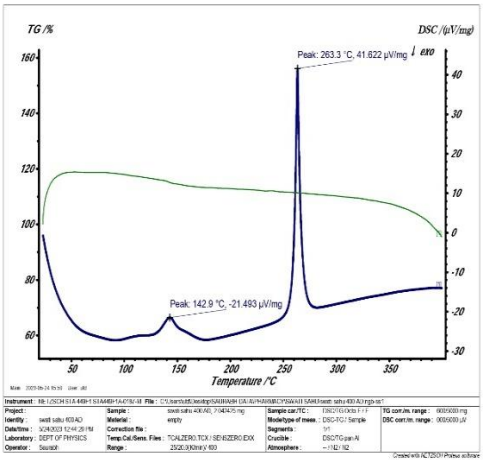
peak was nowhere to be found. The loss of the DS melting peak, broadening of peaks, and shifts in onset temperature and peak shape are all strong indicators of molecular-level interactions between DS and SPC. These alterations suggest that hydrophilic interactions, most importantly hydrogen bonding between the diosmetin hydroxyl group and polar head groups of SPC, contributed to the establishment of a stable molecular complex. The loss of sharp melting transitions and the appearance of broader, less-intense peaks indicate that the drug can be in an amorphous or molecularly dispersed state within the phospholipid matrix.

In addition, the contact with the polar part of the phospholipid is thought to cause the hydrocarbon chains to become mobile, enabling the tail section to change conformation, as evidenced by the thermogram. In aggregate, the DSC information presents definitive proof for the effective formation of the DS–SPC complex, as evidenced by deviations in thermal response indicative of close molecular contact and a possible trend toward increased solubility and bioavailability.



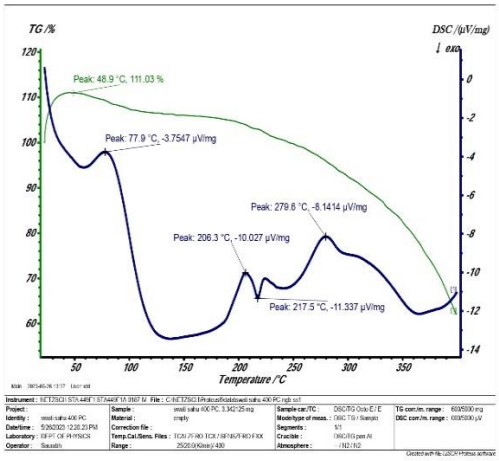
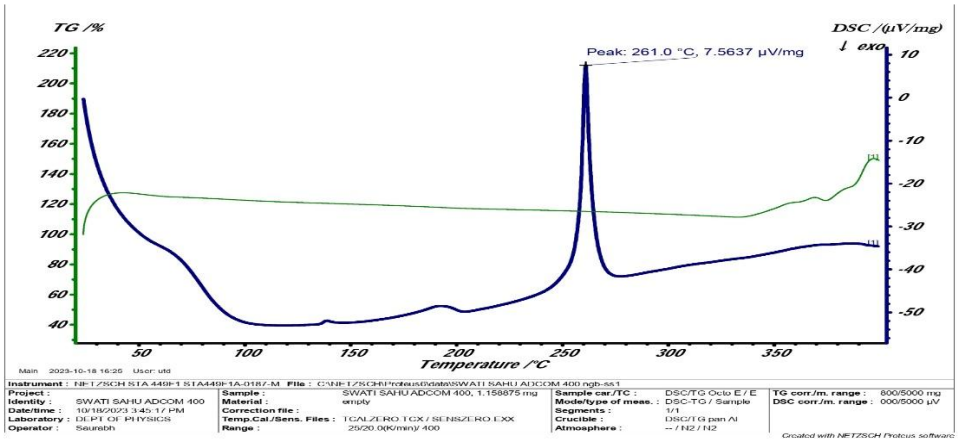


Figure 4: DSC Thermogram A) Diosmetin B) Soyaphosphatidylcholine



C) DS-SPC complex

3.7 X-Ray diffraction (XRD)

The appearance of some vigorous crystalline peaks is indicative of the presence of pure flavonoid in crystalline form. Diosmetin had typical peaks (2θ) at 28, 32,47, 56, 69. Yet, PXRD pattern analysis of the complex illustrated no such notable peaks, which strongly established the molecularly complexed state of flavonoid in the formulation.

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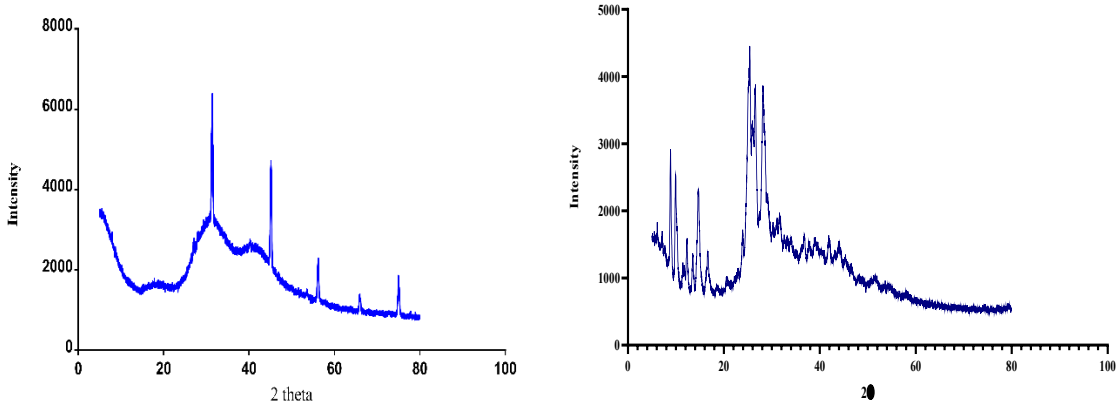
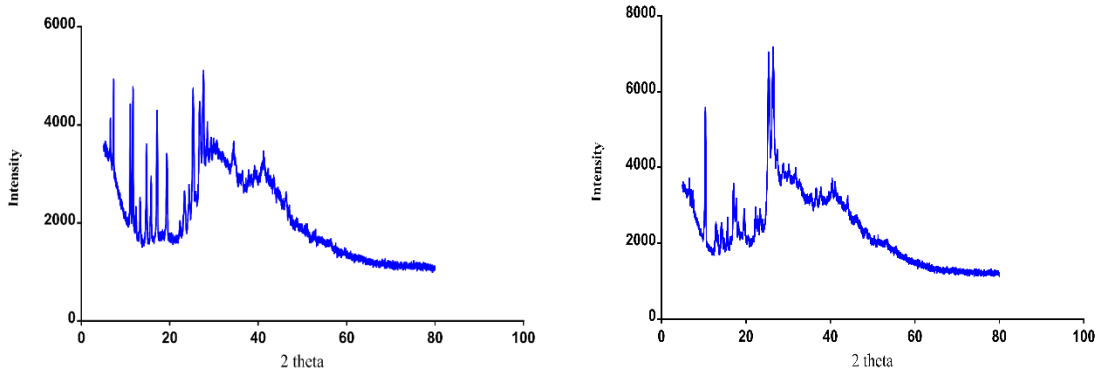


Figure 5: X-ray diffractogram A) Diosmetin B) Soyaphosphatidylcholine



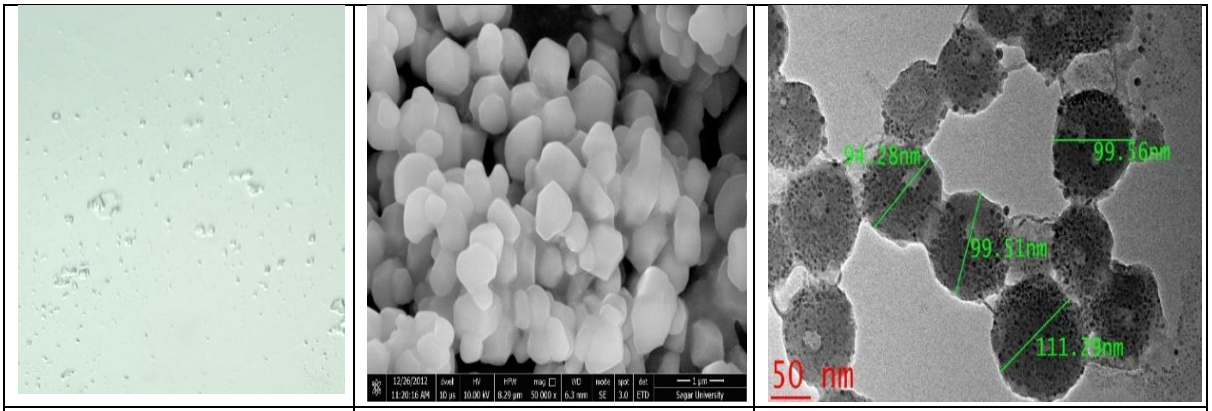
C) DS-SPC complex **D) DS-SPC phytosomes**

3.8 Characterization of the vesicular system

3.8.1Vesicle shape and size

The surface morphology of the exterior surface of the flavonoid–SPC complex was characterized by scanning electron microscopy (SEM). The samples, lightly dusted, were mounted on aluminum stubs and coated with gold using a sputter coater to improve electrical conductivity. SEM micrographs were taken at various magnifications with an accelerating voltage of 10 kV to view the morphological features.

Transmission electron microscopy (TEM) was used for thorough internal structural analysis and estimation of particle size. The solution of the sample was sonicated for 5 minutes, and a few drops of it were put on a 300-mesh carbon-coated copper grid. The grid was left in the air under a lamp to facilitate full evaporation of water. TEM analysis, performed at a suitable accelerating voltage, indicated that the particle size of the phytosomes varied from 94.28 nm to 111.29 nm.



A	B	C

Figure 6: A) Optical microscopy, B) SEM photomicrograph, C) TEM photomicrograph of prepared phytosomes

3.9 Entrapment Efficiency

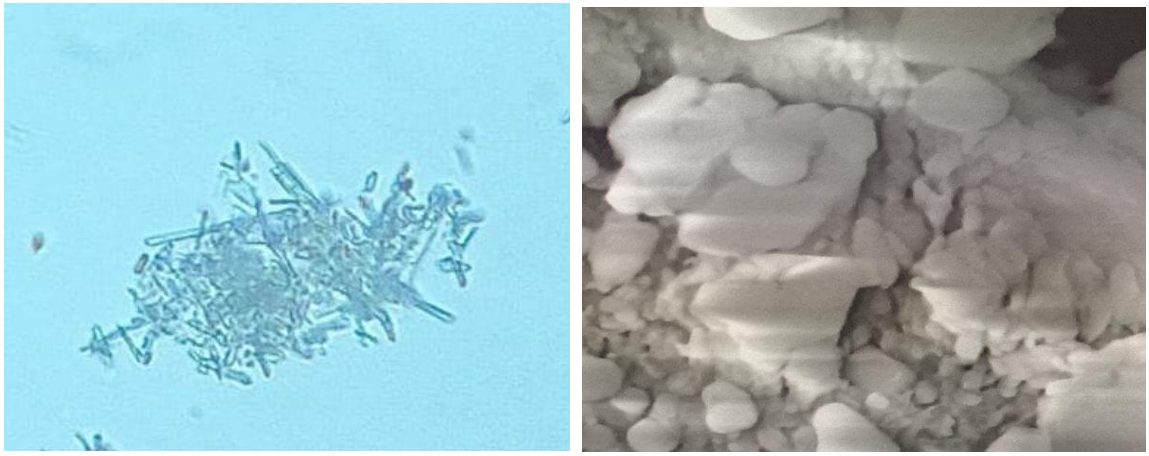
The entrapment effectiveness of flavonoid–SPC complex formulation was 94%, which is indicative of effective loading of the active ingredient into the lipid matrix.

3.10 Stability Studies

Stability analysis performed at 4°C revealed that the phytosome was stable for a short-term storage duration, and no meaningful morphological or pharmaceutical transformation was evident. When the phytosome formulation was stored at room temperature for 90 days, vesicle fusion and aggregation were evident, which indicates a loss of stability at higher temperatures. This emphasizes the need for storage at lower temperatures to ensure the structural and functional stability of the formulation.

Table 1: Effect of storage on the vesicle shape

Storage temperature	Days		
	30	60	90
4 ± 2 °C	Spherical	Spherical	Spherical
25 ± 2 ºC	Spherical	Spherical	Aggregates
40 ± 2 ºC	Aggregates	Aggregates	Aggregates



A **B**

Figure 7: Stability study outcomes of diosmetin Phytosomes, a) optical microscopy, b) SEM photomicrograph after 90 days under room temperature conditions.

3.11 In Vitro Antioxidant Activity

The antioxidant activity of the test samples was evaluated based on their ability to neutralize DPPH, a stable free radical, from a methanolic solution. The freshly prepared DPPH solution is purple, which diminishes in the presence of antioxidant compounds due to the quenching of DPPH radicals. This reaction results in the formation of a colorless product, leading to a decrease in absorbance at 517 nm. In this study, the antioxidant activities of flavonoids and their phytosome formulations were compared with that of the control antioxidant, ascorbic acid. The absorbance of the pure DPPH solution at 517 nm was used as a blank. Both flavonoids and their phytosomes exhibited antioxidant activity, as shown in Table 1, with the phytosome formulations demonstrating higher free radical scavenging activity than free diosmetin, indicating greater antioxidant capacity.

Table 2: Antioxidant activity of the phytoconstituents and their phytosomes in DPPH assay

Conc. of sample → / RSA% ↓	10 (µg/ml)	20 (µg/ml)	30(µg/ml)	40 (µg/ml)	50 (µg/ml)
ASA	71.28	77.81	88.90	98.04	98.38
A	68.35	69.82	70.23	71.82	75.08
B	69.72	71.69	74.88	78.55	82.27

ASA-Ascorbic acid, A-Diosmetin, and B-Diosmetin Phytosomes

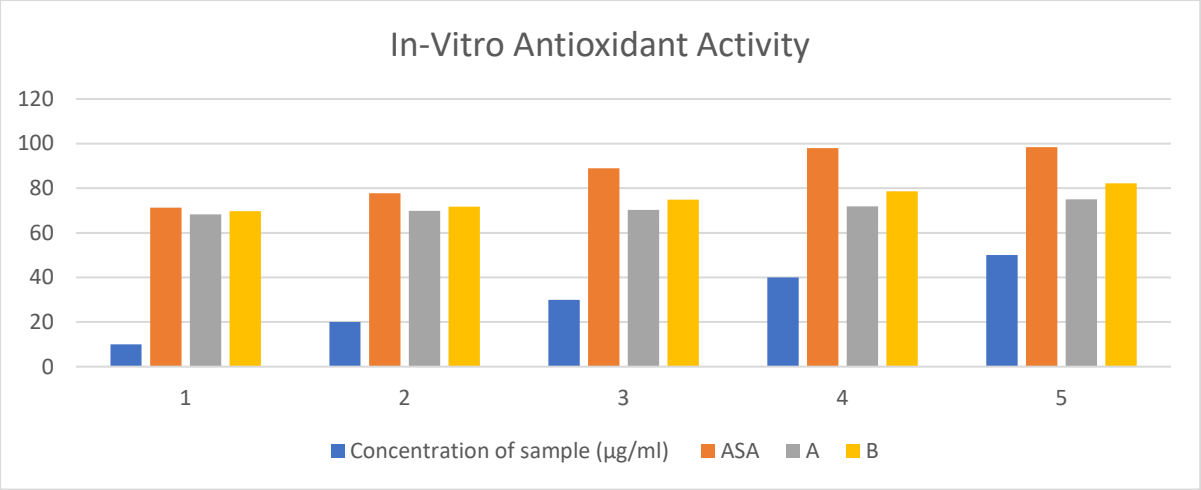


Figure 8: *In Vitro* Antioxidant Activity

3.12 Evaluation of antidiabetic potential

3.12.1 Streptozotocin-induced diabetic model

A single dose of streptozotocin (32.5mg/kg p.o) caused a dramatic elevation in blood glucose in rats, as indicated in Table 2. After 21 daily treatments with diosmetin (20 mg/kg in normal saline) and diosmetin phytosomes (equivalent to 20 mg/kg diosmetin in normal saline), blood glucose was significantly decreased in diabetic rats. On day 21, blood glucose level was 119 ± 13.09 mg/dL in the diosmetin and 101 ± 4.04 mg/dL in the diosmetin phytosomes-treated group. Diosmetin phytosome formulation exerted a more significant glucose-lowering activity than plain diosmetin and showed efficacy comparable to the reference standard, metformin.

Table 3: Blood glucose levels in rats

Groups	Blood glucose level (mg\dl)			
	Initial day	Day 7th	Day 14th	Day 21st
Group 1	109±13.50	89±47.25	118±12.16	115±11.53
Group 2	235±7.76	289±21.07	296±5.03	323±12.05
Group 3	189±32.56	165±20.03	142±21	119±13.09
Group 4	176±21.07	149±21.65	121±13.05	101±4.04
Group 5	210±24.00	171±10.01	135±10.06	107±5.03

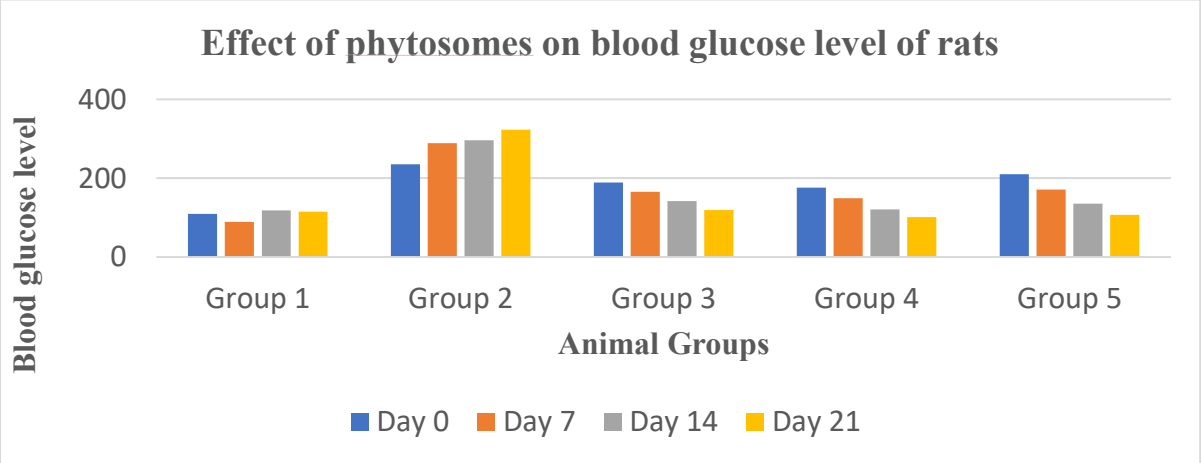


Figure 9: Blood glucose levels in rats

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3.13 Histopathological studies

3.13.1 Effect on the pancreas

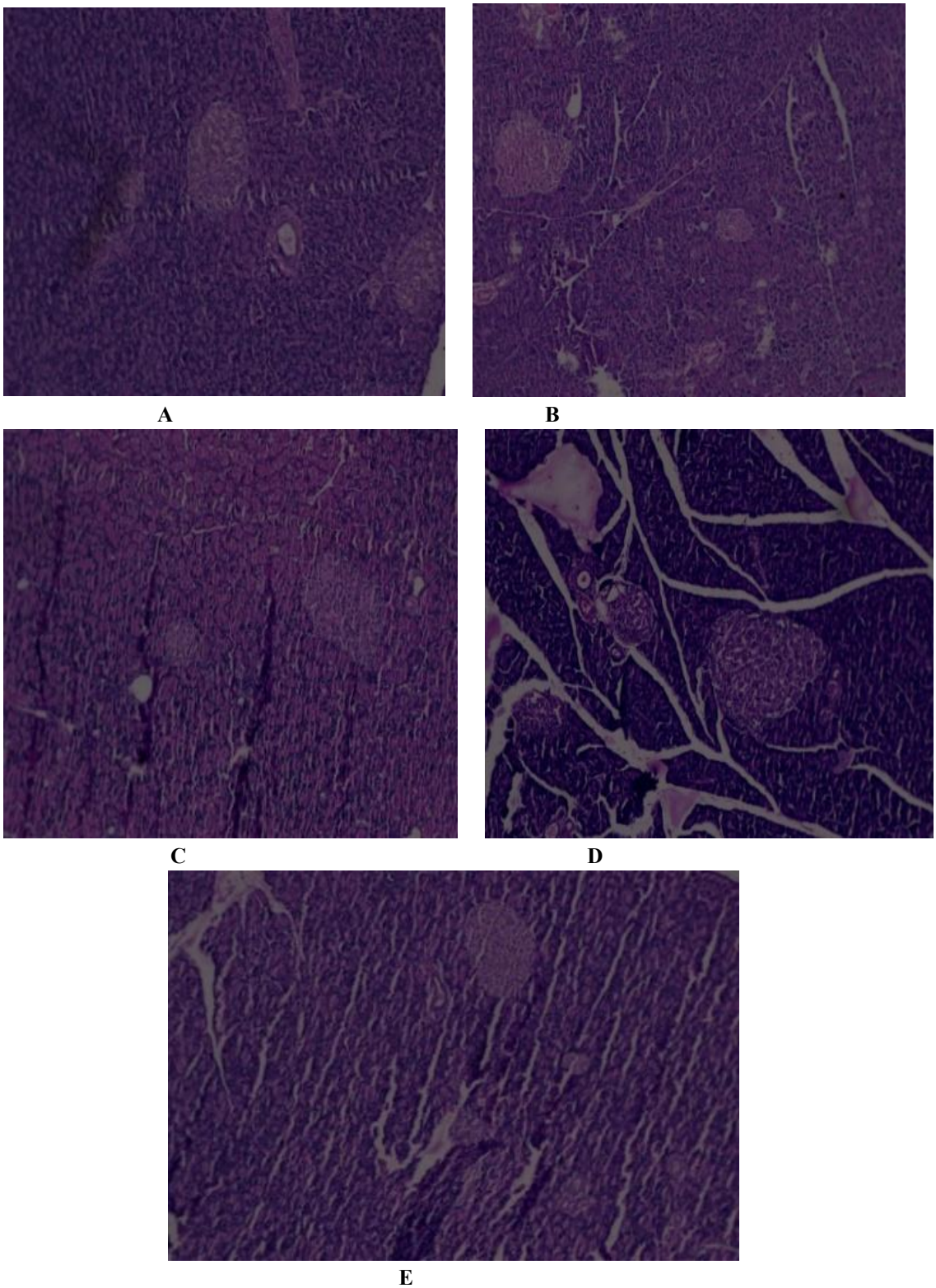

Figure 10: Histopathological changes on pancreas: (A) Control, (B) Streptozotocin-induced group, (C) Diosmetin treated, (D) Diosmetin phytosomes, and (E) Metformin.

Histological assessment of the pancreas demonstrated typical acinar architecture and preserved cellularity of the islets of Langerhans of the normal control group (Figure 7A). Diabetic rats,

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however, had profound destruction of the islets and marked reduction of islet size (Figure 7B). Treatment with diosmetin (Figure 7C), diosmetin phytosomes (Figure 7D), and metformin (Figure 7E) demonstrated restoration towards normal islet structure and cellular number, suggesting protective and regenerative effects on pancreatic tissue. The level of recovery was more pronounced in diosmetin phytosomes compared to plain diosmetin.

3.13.2 Effect on the liver

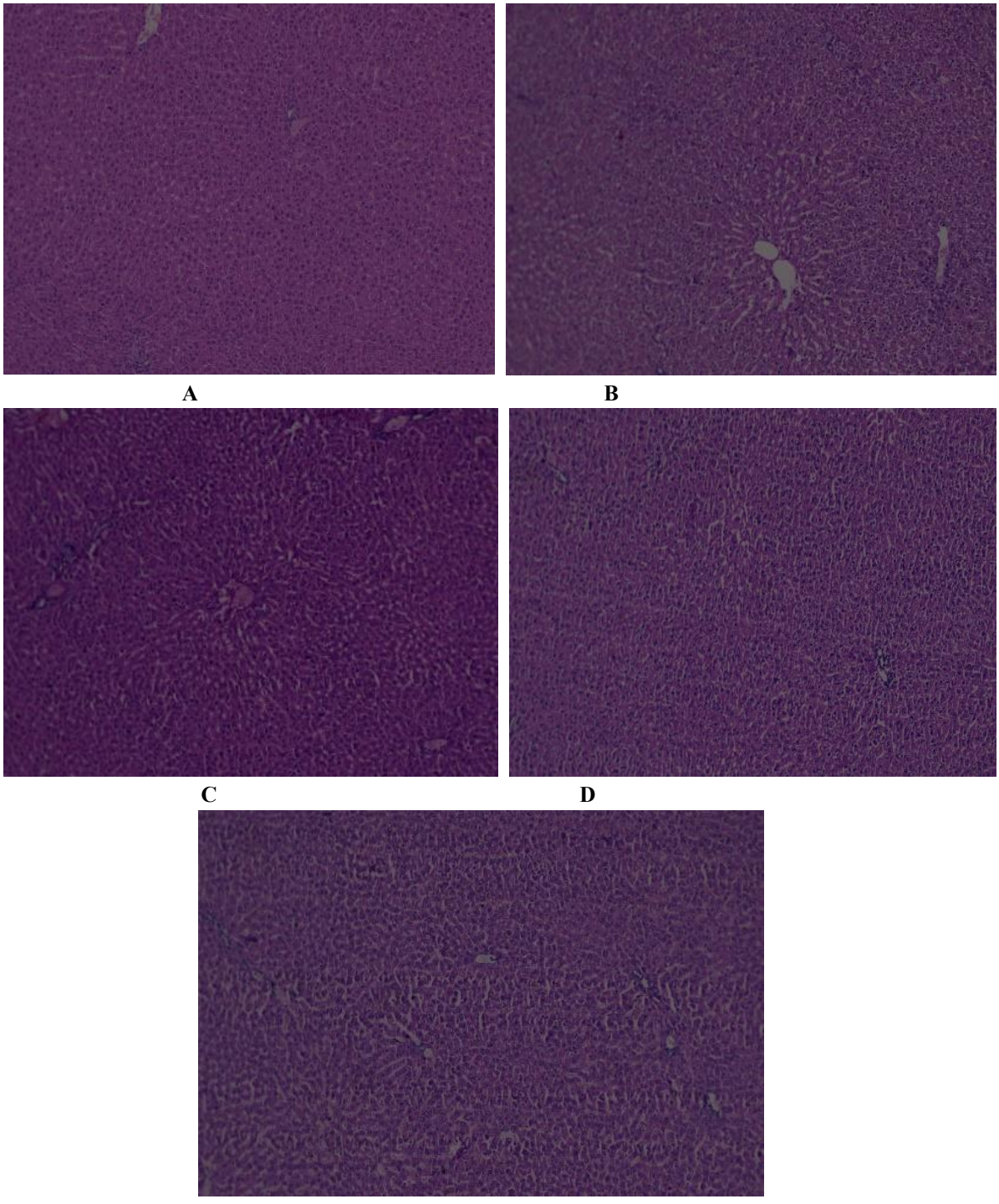

Figure 11: Histopathological changes on liver: (A) Control, (B) Streptozotocin-induced group, (C) Diosmetin-treated, (D) Diosmetin phytosomes, and (E) Metformin.

Histopathological evaluation of the liver tissue of streptozotocin-induced rats demonstrated intense centrolobular necrosis (Figure 8B). Liver sections in rats treated with diosmetin (Figure 8C), diosmetin phytosomes (Figure 8D), and metformin (Figure 8E) indicated healing of hepatic damage as indicated by the restoration of normal hepatic cords and lack of necrotic changes.

Discussion

The results unequivocally show that the phytosome formulation was more rapid and effective in lowering blood glucose levels in streptozotocin-induced diabetic rats than the plain drug. This is a result of the intrinsic limitations of most phytoconstituents, which tend to have poor water solubility and poor absorption due to the lipophilic nature of biological membranes. The latter are made up of a lipid bilayer that impairs the transport of hydrophilic molecules.

In the current study, phytosomes made up of lecithin and cholesterol enhanced drug delivery through increased interaction of the phytoconstituent with the lipid bilayer, thus ensuring effective membrane penetration. The phytosome structure enables the water-soluble active principle to be entrapped in a lipid-compatible covering, which enhances its lipophilicity, improves membrane permeability, and eventually results in improved bioavailability.

These results validate the presumption that the limited oral bioavailability of free phytoconstituents can be considerably enhanced through the application of phytosomal technology to facilitate greater absorption and therapeutic effect.

Conclusion

The current research exemplifies an efficient approach to improving the bioavailability of diosmetin by designing a phospholipid-based phytosome formulation. Enhanced solubility and dissolution profiles are anticipated to enhance pharmacodynamic performance. The results also verify the antidiabetic potential of diosmetin in streptozotocin-induced diabetic models. In conclusion, this study highlights the potential of employing phytosome technology to break biopharmaceutical constraints related to flavonoid compounds and provides a promising platform for enhancing the therapeutic effectiveness of poorly soluble flavonoids.

Conflict of interest

The authors declare no conflict of interest regarding the data and publication of this manuscript.

Animal ethical information

The animal study was approved by the Institutional Animal Ethics Committee (IAEC) by CCSEA (**Vedic/CCSEA/02/26.09.2023**).

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References

1. Datusalia A K, Dora C P, and Sharma S, Acute and chronic hypoglycemic activity of sidatiagii fruits in N5-streptozotocin diabetic rats, Acta Poloniae Pharmaceutica Drug Research, 69: 699-706, 2012.
2. Sashikanth, P. Aravindkumar, and C. Swarupa, “Two-way relation of diabetes mellitus and periodontitis—a review,” Annals and Essences of Dentistry, vol. 4, no. 1, 2012.
3. Almalki, D. A.; Alghamdi, S. A. & Al-Attar, A. M. Comparative study on the influence of some medicinal plants on diabetes induced by streptozotocin in male rats. BioMed Res. Int., 2019:3596287, 2019
4. Garg M, Chaudhary SK, Goyal A, Sarup P, Kumari S, Garg N, Vaid L, and Bhatia S “Comprehensive review on therapeutic and phytochemical exploration of diosmetin: A promising moiety” Phytomedicine Plus 2022, 1-12 https://doi.org/10.1016/j.phyplu.2021.100179.
5. Yang, B.; Dong, Y.; Wang, F.; Zhang, Y. Nanoformulations to Enhance the Bioavailability and Physiological Functions of Polyphenols. Molecules 2020, 25, 4613.
6. Gaikwad, A.R.; Ahire, K.D.; Gosavi, A.A.; Salunkhe, K.S.; Khalkar, A.; Gaikwad Abhijeet, R. Phytosome as a Novel Drug Delivery System for Bioavailability Enhancement of Phytoconstituents and its Applications: A Review. J. Drug Deliv. Ther. 2021, 11, 138–152.
7. Jain, P.K.; Khurana, N.; Pounikar, Y.; Gajbhiye, A.; Kharya, M.D. Enhancement of absorption and hepatoprotective potential through soya-phosphatidylcholine-andrographolide

vesicular system. *J. Liposome Res*., 2013, *23*(2), 110-118.

8. Manral K, Singh AK, Sah V, Development and Characterization of Morin Loaded Phytosomes for its Anti-Oxidant Activity, Journal of Drug Delivery and Therapeutics. 2019; 9(4):30-36 http://dx.doi.org/10.22270/jddt.v9i4.2971
9. Jain, P.K.; Kharya, M.; Gajbhiye, A. Pharmacological evaluation of mangiferin herbosomes for antioxidant and hepatoprotection potential against ethanol-induced hepatic damage. *Drug Devel. Indus. Pharm.*, 2013, *39*(11), 1840-1850.
10. Mahapatra, K. Patil, S., and Gajbhiye A., The progressive journey of phytosomes in herbal-based pharmacotherapeutics, Current Bioactive Compounds, 2020, 853-886.
11. Djekic, L.; Krajišnik, D.; Mićic, Z.; Čalija, B. Formulation and physicochemical characterization of hydrogels with 18β-glycyrrhetinic acid/phospholipid complex phytosomes. *J. Drug Deliv. Sci. Technol*., 2016, *35*, 81-90.
12. Udapurkar, P., Bhusnure, G., and Kamble, R. Diosmin Phytosomes: Development, Optimization and Physicochemical Characterization, 2018 DOI: 10.5530/ijper 52.4s.73
13. Saad M. A, Optimization and Characterization of Cuscuta reflexa Extract Loaded Phytosomes by the Box-Behnken Design to Improve the Oral Bioavailability 2022 doi: 10.5650/jos.ess 21318.
14. Karole, S, Gautam, GK and Gupta, S, 'Preparation and evaluation of phytosomes containing ethanolic extract of leaves of Bombax ceiba for hepatoprotective activity', The Pharma Innovation Journal 2019; 8(2): 22-26.
15. Anwar E, Farhana N. Formulation and evaluation of phytosome-loaded maltodextrin-gum Arabic microsphere system for delivery of Camellia sinensis extract. Journal of Young Pharmacists. 2018, 56.
16. Furman, B.L. 2015 Streptozotocin-induced diabetic models in mice and rats. *Curr. protocol.pharmacol* 70:5.47 1-5,47.20. doi:10.1002/0471141755.ph0547s54.
17. Pradeepa M, Narayanan V, Cherukuri S, Nivetha R, Ganesan S, Anitha P, and Lavakumar V. A Review on Phytosomes, Importance, and Their Applications. International Journal of Phytopharmacology, 2018; 9(1): 22-28. DOI: http://dx.doi.org/10.21276/ijp.2018.9.1.4
18. Sheoran S, Arora S. Singh, H. Kumar, A. and Vuree S., "Characterisation, development and validation of UV spectrophotometric technique for the determination of Diosmetin in bulk and nanoformulations", *Results in Chemistry* (2023), doi: https://doi.org/10.1016/j.rechem.2023.100 972

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