Enhancing Hepatoprotective Efficacy Of Myricetin Through Nano-Liposomal Drug Delivery Systems

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ABSTRACT

Myricetin, is a kind of naturally occurring polyhydroxy flavonoid, exhibits strong antioxidant, anti-inflammatory and hepatoprotective capabilities. This research aims to develop of a nano liposomal formulation of myricetin to enhance its physical and chemical characteristics as well as therapeutic potential for hepatoprotection. Liposomes were prepared using the lipid layer hydration method employing soya phosphatidylcholine (SPC), cholesterol (CHL), and butylated hydroxyanisole (BHA). Pre formulation compatibility studies were performed using Fourier Transform Infrared (FTIR) spectroscopy, and solubility studies were carried out in various solvents and physiological media. Nano-liposomal resulted in a remarkable improvement in aqueous solubility, achieving approximately an 11-fold increase in water and a 10-fold increase in PBS (pH 7.4) compared to plain myricetin. The formulation exhibited high encapsulation efficiency (86.7–87.6%) and satisfactory percentage yield (78.5–80.2%), demonstrating reproducibility and suitability for further development. Histopathological evaluation confirmed superior hepatoprotective activity of formulated myricetin compared to plain myricetin and the standard drug (5-FU). Overall, this study provides a comprehensive scientific basis for advancing Nano-liposomal myricetin into further pharmacological investigations and potential therapeutic applications.

Keywords: Myricetin, Liposomes, Nanoformulation, Hepatoprotective activity, FTIR, Solubility studies.

1. INTRODUCTION

Hepatic diseases remain a significant worldwide health concern, often arising from oxidative stress, drug induced toxicity, fat accumulation and metabolic disorders [1]. Natural flavonoids have attracted considerable attention for their hepatoprotective potential largely due to their strong antioxidant and anti-inflammatory effects [2]. Flavonoids are plant-derived compounds that are generally safe and exhibit a wide range of beneficial biological effects. However, even with their strong pharmacological potential, their use in the clinical application of flavonoids has been limited by their poor water solubility and low bioavailability, limited stability in the body and insufficient delivery at target tissues. Therefore, encapsulating these phytocomponents in suitable nano-vehicles is essential to enhance their therapeutic effectiveness. One such flavonoid, Myricetin, a polyhydroxy flavonol found in fruits, vegetables, and medicinal plants, has demonstrated promising hepatoprotective, antioxidant, and cytoprotective properties. Myricetin is a potent liver-protecting agent. Its effectiveness stems primarily from its unique structure, which allows it to combat the "multiple hits" of liver disease: oxidative stress, inflammation, and fat accumulation.[3] Myricetin is one of the strongest flavonoid antioxidants because of its three hydroxyl groups on the B-ring. It activates the Nrf2 signalling pathway, which is the body's internal "thermostat" for antioxidant defense. This leads to an increase in protective enzymes like Superoxide

Dismutase and Glutathione, which neutralize free radicals before they can damage liver cells hepatocytes. Myricetin downregulates transcription factors like PPAR-γ and SREBP-1c, which are responsible for creating new fat in the liver. myricetin has low bioavailability—meaning the body has a hard time absorbing and using it when taken orally. Current research is focusing on nano formulations (like liposomes) to package the molecule so it can bypass digestive breakdown and reach target tissues more effectively [4].

Despite these benefits, its therapeutic application is hindered by poor solubility and limited bioavailability.

Nanotechnology based drug delivery systems, particularly liposomes, offer an effective strategy to overcome these limitations [5]. Liposomes are biocompatible vesicular systems capable of encapsulating both hydrophilic and lipophilic drugs, improving drug stability, solubility, and targeted delivery. The present work aims to formulate myricetin loaded liposomes and perform essential pre formulation and solubility studies as a preliminary step toward evaluating their hepatoprotective efficacy.

The reason that this compound works lies in how it is structured, and therefore, how it can fight against the three components that contribute to liver disease: lipid accumulation, oxidative stress, and inflammation. The compound will activate the Nrf2 signaling pathway, which is essentially a thermostat for the body's antioxidant defense system.[6] In addition protective enzymes such as superoxide dismutase and glutathione, activating Nrf2 causes liver cells to neutralize the free radical molecules. In essence, the compound activates the Nrf2 signaling pathway, a thermostat for antioxidant defense in the body. The compounds will also down-regulate transcription factors PPAR-γ and SREBP-1c, which are responsible for the creation of new fat in the liver.

Myricetin has poor bioavailability, or in other words, the body has difficulty digesting and utilizing it when administered orally. Current research is primarily focus on developing nano formulations to enclose the molecule to protect it from digestive degradation and facilitate its delivery to the target tissue.

It is possible to overcome the problems associated with myricetin's poor bioavailability by nano formulating drugs, specifically liposome-based drug delivery systems [7]. In addition to enhancing drug stability, solubility, and targeted delivery, liposomes are biocompatible, vesicular systems capable of encapsulating hydrophilic or lipophilic drugs. Using liposome-based drug delivery systems, as a means of circumventing the problems caused by myricetin's poor bioavailability, represents a viable approach. Using liposomes, drugs can be encapsulated in either hydrophilic or lipophilic form, improving drug stability, solubility, and targeted delivery.

2. OBJECTIVE OF THE STUDY

The study focuses on developing nano-liposomal myricetin using the lipid layer hydration approach and evaluating its overall potential as an enhanced drug delivery system. The study involved investigating drug–excipient compatibility through FTIR spectroscopy, evaluating the solubility properties of myricetin in various solvents and physiological conditions, and determining formulation characteristics such as the encapsulation efficiency, percentage yield, and batch reproducibility of the prepared formulations. This research aims to provide a solid preclinical basis for improving the hepatoprotective efficacy of myricetin using nanoliposomes.

MATERIALS AND METHODS

3.1 Materials

Myricetin was used as the active pharmaceutical ingredient. Soya phosphatidylcholine (SPC), cholesterol (CHL), phosphatidylethanolamine (PE), and butylated hydroxyanisole (BHA) were used for liposome preparation. All solvents and reagents were of analytical grade.

3.2 Preparation of Liposomal Myricetin

Liposomes were prepared using the lipid layer hydration method. Accurately weighed quantities of SPC, CHL, and BHA (1% w/v) were dissolved in chloroform in a 250 mL round bottom flask and mixed thoroughly by vigorous shaking. The organic solvent was evaporated using a rotary vacuum evaporator fitted an A3S aspirator, operating at 150 rpm and maintained at 37°C in a circulating water bath. The formed thin lipid film was further dried overnight in a vacuum desiccator to ensure complete removal of any residual solvent.

Myricetin solution (1 mg/mL) prepared in deionized water was added to the flask containing the dried lipid film. Hydration was carried out at 60°C using a rotary vacuum evaporator at 100 rpm until complete dispersion of the lipid film into the aqueous phase. The dispersion was sonicated using a bath type sonicator at approximately 30 kHz while maintaining the same temperature. After sonication, the formulation was allowed to stand at room temperature for 1 hour to facilitate vesicle formation and subsequently stored overnight at 4°C. The liposomal dispersion was centrifuged at 16,000 rpm for 1 hour, and the obtained sample was lyophilized for further studies.

For PE conjugated liposomes, weighed amounts of SPC, CHL, and BHA were dissolved in a chloroform:methanol mixture (3:1) and processed using the same procedure described above.

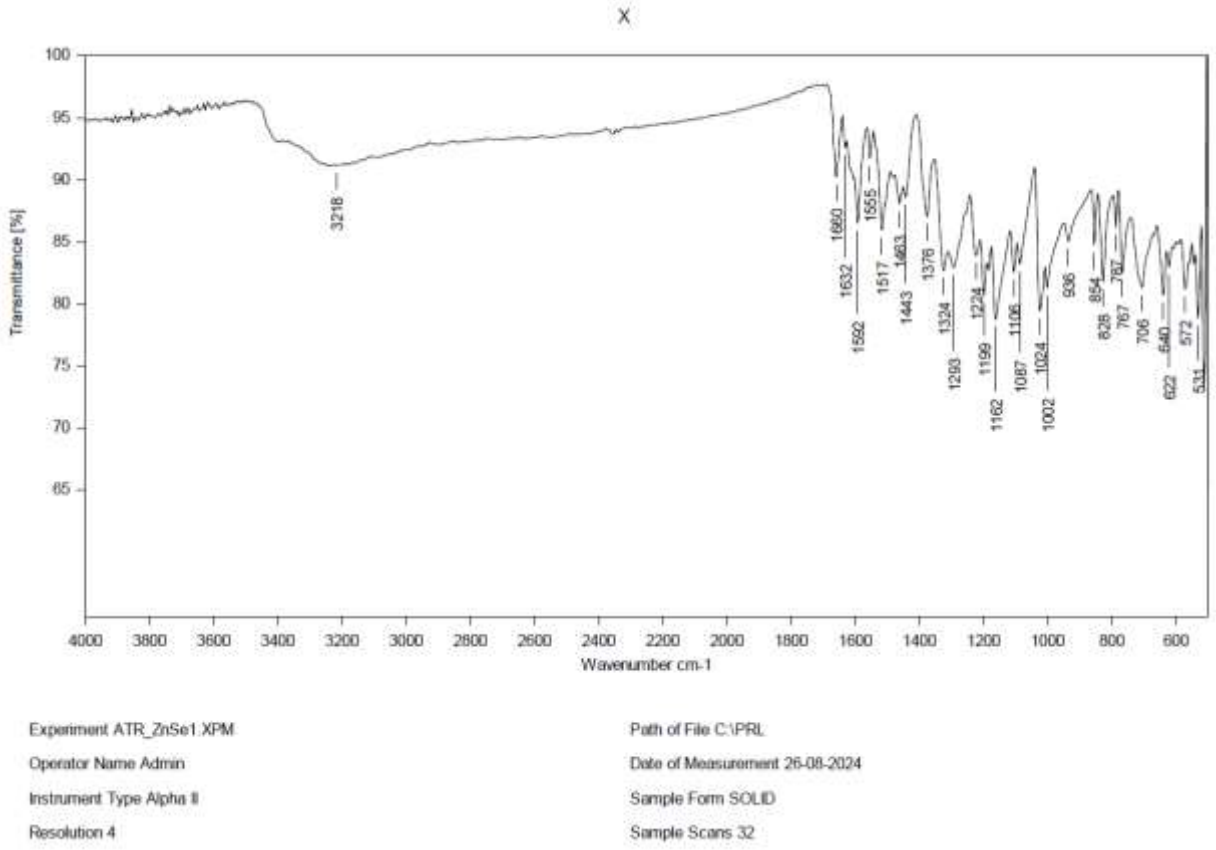
3. RESULTS

4.1 Pre formulation Studies by FTIR

a. FTIR Analysis of Plain Myricetin:

Fourier Transform Infrared (FTIR) spectroscopy was carried out to identify the characteristic functional groups of pure myricetin and to confirm its chemical integrity prior to formulation. The FTIR spectrum of myricetin was recorded using an Alpha II FTIR spectrometer in the wavenumber range of 4000–400 cm^{-1} using the KBr pellet method.

The FTIR



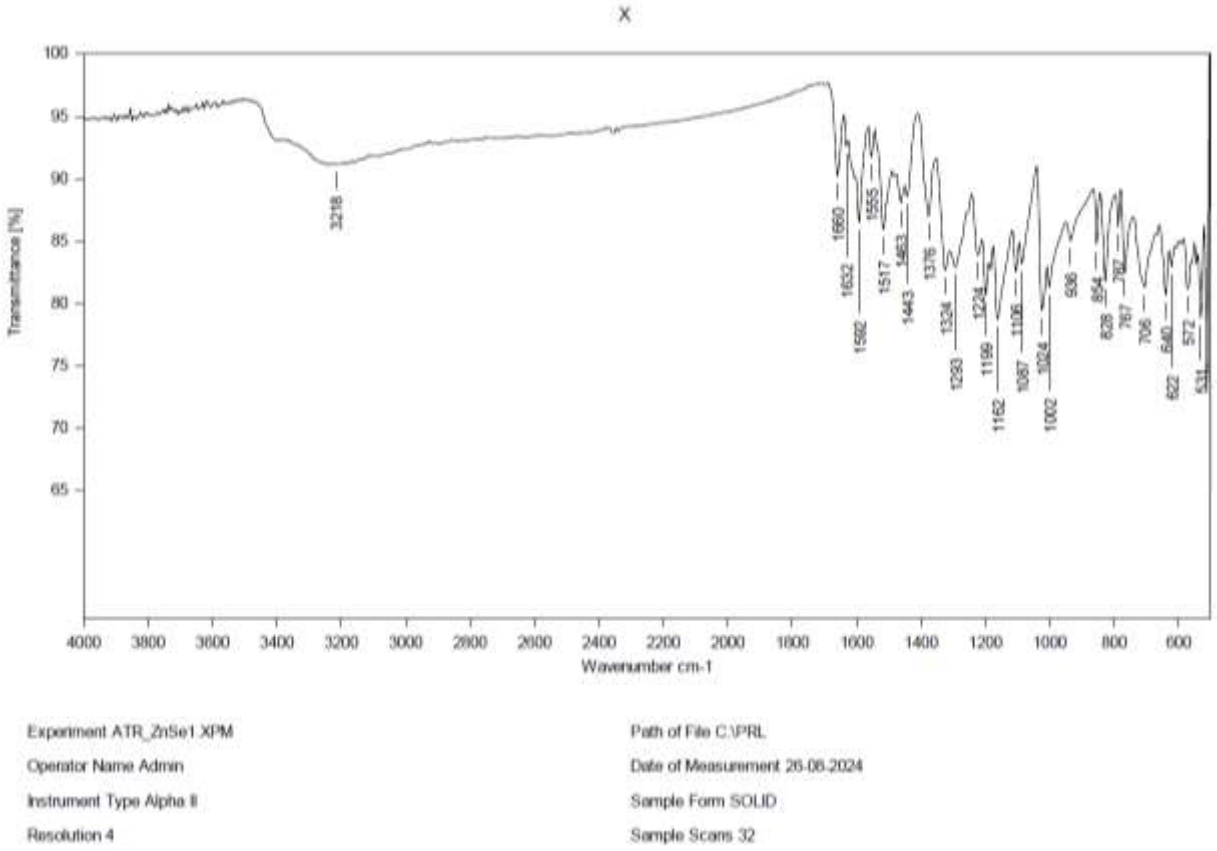
spectrum of plain myricetin displayed prominent peaks corresponding to phenolic hydroxyl (–OH), aromatic C=C, carbonyl (C=O), and ether (C–O–C) functional groups, which are characteristic of the flavanol structure of myricetin. The broad absorption band observed in the region of 3400–3200 cm^{-1} confirms the presence of multiple hydroxyl groups, which are responsible for the antioxidant and biological activity of myricetin.

The strong absorption peak around 1650 cm^{-1} corresponds to the carbonyl stretching vibration, confirming the presence of the flavone backbone. Aromatic ring vibrations observed in the region of 1600–1500 cm^{-1} further confirm the integrity of the benzene ring system. The absence of any additional or shifted peaks indicates that the drug is chemically pure and has not undergone degradation.

The FTIR analysis of plain myricetin confirmed the presence of all characteristic functional groups, indicating the chemical integrity and purity of the drug. These results establish myricetin as suitable for further formulation development and compatibility studies with excipients.

b. FTIR Analysis of Formulated Myricetin (MyNL)

Fourier Transform Infrared (FTIR) spectroscopy was carried out to evaluate the chemical compatibility of myricetin with formulation excipients and to confirm the structural integrity of the drug after formulation. The FTIR spectrum of formulated myricetin (MyNL) was recorded using an Alpha II FTIR spectrometer in the wavenumber range of 4000–400 cm^{-1} employing the ATR (ZnSe crystal) technique.



The FTIR spectrum of formulated myricetin showed all characteristic functional group peaks of pure myricetin, including hydroxyl (–OH), aromatic C=C, and carbonyl (C=O) groups, with minor shifts in peak positions and reduced intensity. These changes are attributed to physical interactions such as hydrogen bonding and molecular encapsulation within the lipid matrix rather than chemical incompatibility.

The presence of additional peaks corresponding to aliphatic C–H stretching, ester carbonyl, and phosphate groups confirms the successful incorporation of lipid excipients such as SPC, CHL, and PE in the formulation. Importantly, no new peaks or disappearance of characteristic myricetin peaks were observed, indicating that the drug remained chemically stable during formulation and lyophilization.

FTIR analysis of formulated myricetin confirmed the retention of all characteristic functional groups of myricetin with no evidence of chemical interaction with excipients. The results demonstrate excellent drug–excipient compatibility and confirm the structural stability of myricetin in the formulated system.

4.2 Solubility Studies

FTIR spectra of pure myricetin and its physical mixtures with excipients revealed the presence of characteristic functional group peaks without significant shifts or disappearance, indicating the absence of chemical incompatibility between the drug and formulation components. Solubility studies confirmed the poor aqueous solubility of myricetin and highlighted the need for a nano delivery system to enhance its dissolution characteristics. The liposomal formulation approach is expected to significantly improve the solubility, stability, and bioavailability of myricetin, thereby enhancing its hepatoprotective potential.

Solubility studies were carried out to determine the solubility profile of myricetin in various solvents and dissolution media, which would aid in the selection of appropriate dissolution and diffusion fluids for drug release and pharmaceutical studies. The solubility of myricetin was determined by the incremental addition of 100 mg of the drug sample to different solvents until saturation was achieved.

Myricetin solubility was evaluated in a range of solvents, including distilled water, methanol, ethanol, chloroform, 0.1 N hydrochloric acid (HCl), phosphate-buffered saline (PBS), and dimethyl sulfoxide (DMSO). Each mixture was agitated continuously at room temperature until equilibrium was reached. The solutions were then visually examined to assess solubility.

Table 1: Solubility of Plain and Nano-liposomal Myricetin

Formulation	Solubility in Water (µg/mL)	Solubility in PBS pH 7.4 (µg/mL)
Plain myricetin	8.6 ± 1.2	12.4 ± 1.6
Nano liposomal myricetin	96.8 ± 6.4	128.3 ± 8.1

The solubility of nano liposomal myricetin was markedly higher than that of plain myricetin in both aqueous media. Encapsulation into nanoliposomes resulted in approximately 11-fold enhancement in water and 10-fold enhancement in PBS (pH 7.4), demonstrating the effectiveness of the nano liposomal system in improving aqueous solubility.

Table 2: Solubility of Myricetin in Different Solvents

Solvent	Solubility (mg/mL)	Solubility Character
Distilled water	0.02 ± 0.004	Very slightly soluble
0.1 N HCl	0.05 ± 0.006	Very slightly soluble
PBS (pH 7.4)	0.03 ± 0.005	Very slightly soluble
Ethanol	1.80 ± 0.12	Sparingly soluble
Methanol	2.50 ± 0.15	Sparingly soluble
Chloroform	0.90 ± 0.08	Slightly soluble
DMSO	50.00 ± 1.25	Freely soluble

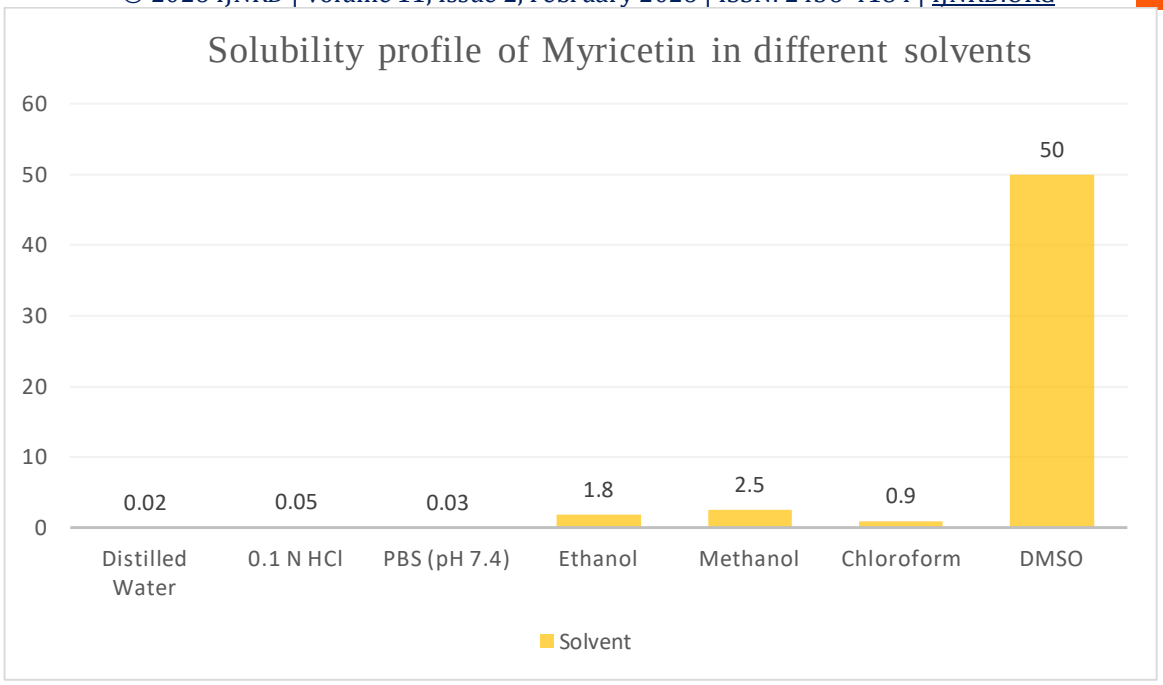


Figure 1: Solubility profile of Myricetin in different solvents

Myricetin exhibited extremely poor solubility in aqueous media, with solubility values below 0.05 mg/mL in distilled water, 0.1 N HCl, and PBS (pH 7.4). Such low aqueous solubility may significantly limit its oral bioavailability and therapeutic effectiveness.

In contrast, myricetin showed substantially higher solubility in organic solvents, with maximum solubility observed in DMSO, followed by methanol and ethanol. These findings confirm the hydrophobic nature of myricetin.

Notably, nano liposomal encapsulation led to a significant enhancement in aqueous solubility, indicating improved dispersion of myricetin in water and PBS. This improvement can be attributed to the lipid bilayer structure of nanoliposomes, which facilitates the incorporation of poorly water-soluble drugs and enhances their apparent solubility.

Overall, the solubility study confirms that myricetin is poorly soluble in aqueous media, thereby justifying the need for advanced formulation strategies such as nano liposomal encapsulation to improve its solubility and potential bioavailability.

4.3 Yield and Encapsulation Efficiency of Nano-liposomal Myricetin

The objective of this study was to determine the percentage yield and encapsulation efficiency (EE%) of myricetin-loaded nanoliposomes (MyNL) in order to evaluate the effectiveness of the liposomal formulation process.

a. Determination of plain Myricetin (C_1)

An aliquot of myricetin-loaded nanoliposomes (MyNL, 0.5 mL) was diluted to 10 mL with phosphate buffer saline (PBS, pH 7.4). The dispersion was centrifuged at 8000 rpm for 30 min to separate the free (unencapsulated) myricetin. The supernatant was collected, and the absorbance was measured at 370 nm using a UV–Visible spectrophotometer. The free myricetin concentration was calculated from the previously established calibration curve and expressed as C_1.

b. Determination of total Myricetin (C_2)

Another 0.5 mL aliquot of MyNL was treated with 4.5 mL of anhydrous ethanol to disrupt the liposomal membrane and release the encapsulated drug. The solution was then diluted to 10 mL with PBS and centrifuged at 8000 rpm for 30 min. The absorbance of the supernatant was measured at 370 nm, and the total myricetin concentration was calculated as C_2.

Encapsulation Efficiency Calculation

Encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation Efficiency (\%)} = \left(\frac{C_2 - C_1}{C_2}\right) \times 100$$

Where:

C_1 = concentration of free myricetin (µg/mL)

C_2 = total myricetin concentration after liposome disruption (µg/mL)

Table 3: Encapsulation Efficiency and Percentage Yield of Myricetin-Loaded Nanoliposomes (MyNL) Across Different Batches

Batch	C_1 (Free drug, µg/mL)	C_2 (Total drug, µg/mL)	Encapsulation Efficiency (%)	Percentage Yield (%)
MyNL-1	12.4 ± 0.9	96.2 ± 2.1	87.1 ± 1.6	78.5 ± 2.4
MyNL-2	13.1 ± 1.1	98.6 ± 1.8	86.7 ± 1.9	80.2 ± 1.8
MyNL-3	11.8 ± 0.8	95.4 ± 2.5	87.6 ± 1.4	79.6 ± 2.1

EE% = (96.2−12.4)/96.2×100 = 87.1%

Myricetin-loaded nanoliposomes revealed effective drug entrapment within the lipid bilayer, with encapsulation efficiency varying from 86.7% to 87.6%. Myricetin's lipophilic properties, which facilitate its integration into the phospholipid membrane, are make up for the high encapsulation efficiency.

The formulation's yield percentile varied from 78.5% to 80.2%, indicating minimal material loss during preparation and elimination and procedure uniformity. The analytical method's specificity has been confirmed by the unloaded nanoliposomes having lack of absorbance at 370 nm.

The prepared myricetin-loaded nanoliposomes exhibited high encapsulation efficiency and satisfactory yield, confirming the suitability of the liposomal formulation approach for improving the delivery of poorly water-soluble myricetin.

4.4 Histopathological evaluation:

At the end of week 15, liver tissues from all experimental groups were collected, fixed in 10% neutral buffered formalin, processed by paraffin embedding (FFPE), sectioned at 4–5 µm thickness, and stained with haematoxylin and eosin (H&E). Sections were examined under light microscopy for architectural and cellular alterations.

Histopathological changes were evaluated using a quantitative scoring system by a blinded pathologist.

Table 4: Quantitative Histopathological Scoring System for Assessment of Hepatic Tissue

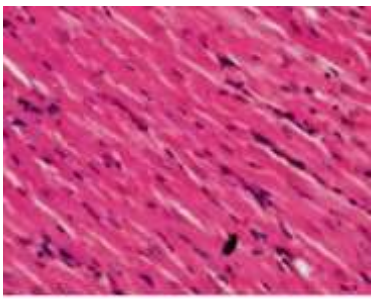
Score	Severity
0	Normal
1	Mild
2	Moderate
3	Severe

Parameters Assessed

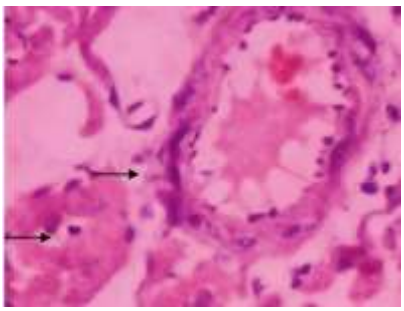
- Hepatic architectural distortion
- Hepatocellular degeneration
- Inflammation
- Necrosis
- Vascular / sinusoidal congestion
- Atypia / neoplastic changes

Experimental Groups

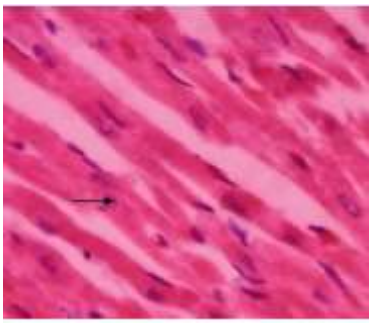
- Normal Control
- DEN-Induced Control
- DEN + Plain Myricetin (M)
- DEN + Formulated Myricetin (FM)
- DEN + Standard Drug (5-Fluorouracil, 5-FU)



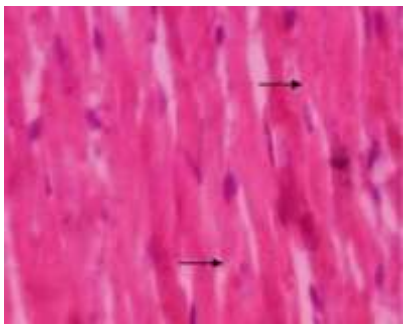
Healthy Normal Control



Mild



Moderate



Severe

Table 5: Quantitative Histopathological Scores (Mean ± SD)

Parameter	Normal Control	DEN Control	Plain Myricetin	Formulated Myricetin	5-FU
Architectural distortion	0.2 ± 0.1	2.6 ± 0.3*	1.4 ± 0.2#	0.6 ± 0.1#†	1.8 ± 0.3#
Hepatocellular degeneration	0.3 ± 0.1	2.8 ± 0.2*	1.6 ± 0.3#	0.7 ± 0.2#†	1.9 ± 0.3#
Inflammation	0.1 ± 0.1	2.5 ± 0.3*	1.3 ± 0.2#	0.5 ± 0.1#†	1.6 ± 0.2#
Necrosis	0.0 ± 0.0	2.4 ± 0.3*	1.2 ± 0.2#	0.4 ± 0.1#†	1.5 ± 0.3#
Vascular congestion	0.4 ± 0.1	2.2 ± 0.2*	1.6 ± 0.3#	1.0 ± 0.2#†	1.9 ± 0.3#
Atypia / neoplasia	0.0 ± 0.0	0.6 ± 0.2*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

* $p < 0.001$ vs Normal Control

# $p < 0.05$ vs DEN Control

† $p < 0.05$ vs Plain Myricetin and 5-FU

Statistical Analysis:

The encapsulation efficiency (%) and percentage yield (%) of myricetin-loaded nanoliposomes (MyNL) across different batches were analysed using one-way analysis of variance (ANOVA) to evaluate batch-to-batch variability. Prior to ANOVA, data were assessed for normal distribution and homogeneity of variance. A p-value < 0.05 was considered statistically significant.

Parameter	Mean ± SD	Statistical Test	p-value
Encapsulation Efficiency (%)	87.13 ± 0.45	One-way ANOVA	> 0.05
Percentage Yield (%)	79.43 ± 0.86	One-way ANOVA	> 0.05

Histological examination revealed preserved hepatic architecture in the Normal control group with no signs of necrosis or inflammation. The DEN-induced group showed extensive hepatic damage, including periportal inflammation, hepatocellular degeneration, focal necrosis, and vascular congestion. Treatment with plain myricetin (Myr) mitigated these lesions but mild congestion and cellular degeneration persisted. In contrast, the formulated nano-liposome myricetin (NLMyr) group displayed near-complete restoration of hepatic architecture with minimal inflammatory or necrotic changes, along with significantly lower histological scores compared to both plain myricetin and 5-FU treatment groups.

4. DISCUSSIONS

According to the current study, myricetin physiochemical properties and overall therapeutic efficacy are significantly improved when it is encapsulated in nano-liposomes markedly. One of the main challengess to myricetin's hepatic therapeutic efficacy is its poor solubility in water and physiological media. By enhancing the dispersion of hydrophobic drug molecules within their lipid bilayer structure, nanoliposomes significantly enhance apparent solubility.

FTIR analysis confirmed that myricetin retained its structural integrity during formulation and showed no evidence of chemical interaction with lipid excipients. This increased bioactivity can be attributed to

improved solubility, more efficient distribution within tissues, and protection against degradation. This study's efficiency of encapsulation (86–88%) is significantly higher and consistent across batches. Myricetin's lipophilic nature, which promote entrapment within the lipid membrane, are contributing to this effectiveness. The proposed method's dependability is further evidenced by the percentage yield values, which show minimal processing loss.

The solubility enhancement achieved through nano-liposome of myricetin has important implications for bioavailability. Histopathological evaluation demonstrated that nano-liposomal myricetin provided substantially greater hepatoprotection than plain myricetin. Improved solubility, better tissue distribution, and degradation protection are responsible for this enhanced bioactivity.

5. CONCLUSION

The current investigation effectively outlines the creation of a myricetin nano-liposomal formulation with substantially improved solubility, high encapsulation efficiency, and encouraging hepatoprotective effects. While solubility studies showed a tenfold improvement in aqueous media, FTIR verified drug integrity and compatibility. When compared to the standard medication and plain myricetin, histopathology reported therapeutic results.

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