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(RESEARCH ARTICLE)

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Development and characterization of *Tinospora cordifolia* extract-loaded SLNs for the treatment of autoimmune hepatitis

Savisa Yadav [1,*], Pooja Rani [2], Kumari Shanno [3], Raman Kumari [4], Shamim [5], Tarmeen Ali [5], Nitin Chandrakant Mohire [6], Gita Nitin Mohire [7] and Ravindra Bhimraj Laware [8]

[1] *Department of Pharmacy, BBDIT, Duhai, Ghaziabad Uttar Pradesh, India.*
[2] *Department of Pharmacy, Krishna Institute Bijnor, Near By -6 milestone Golbhag Chauraha Noorpur Road Bijnor (U.P) 246701, India.*
[3] *Department of Pharmacy, Bansthali Vidyapith, Tonk Road Niwaru – 304022, India*
[4] *Department of Pharmacy, Maharishi Markendeshwar College of Pharmacy, Mullana, Ambala, Haryana, India.*
[5] *IIMT College of Medical Sciences, IIMT University, Ganga Nagar, Meerut, Uttar Pradesh, India – 250001, India.*
[6] *Department of Pharmacy, Shivajirao S Jondhle College of Pharmacy, Asangaon, Thane Maharashtra. 421601, India.*
[7] *Department of Pharmacy, Navsahyadri Institute of Pharmacy, Naigaon, Nasarapur, Pune, Maharashtra 412213, India.*
[8] *College of Pharmaceutical Sciences, PIMS (DU), Loni (Bk)Tal Rahata, Dist. Ahmednagar, Maharashtra, India.*

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Abstract

Solid Lipid Nanoparticles (SLNs) have emerged as promising drug delivery systems with the potential to enhance the therapeutic efficacy of bioactive compounds. In this study, *T. Cordifolia* extract-loaded SLNs were developed and characterized for their application in the treatment of autoimmune hepatitis. *T. Cordifolia*, known for its immunomodulatory and anti-inflammatory properties, was chosen as the active ingredient. The formulations were systematically evaluated for drug-excipient compatibility, particle size, zeta potential, drug loading efficiency, drug release kinetics, encapsulation efficiency, in-vitro release, and stability. The results indicated "No Change" in drug-excipient compatibility, ensuring the formulation's stability. The SLNs exhibited nanoscale particle sizes (159.30 nm to 172.12 nm) with narrow size distributions, facilitating consistent drug delivery. Negative zeta potentials (-28.28 mV to -35.44 mV) indicated colloidal stability. High drug loading efficiencies (up to 32.23%) and controlled drug release kinetics were observed, suggesting the potential for sustained and targeted drug delivery. Encapsulation efficiencies of up to 83.41% highlighted efficient drug loading within the SLNs. In-vitro release studies revealed that SLN2 and SLN4 exhibited superior drug release profiles compared to other formulations. These findings indicate the potential of these formulations for controlled drug delivery. In-vivo efficacy studies in murine models of autoimmune hepatitis are recommended to assess the therapeutic benefits of T. Cordifolia extract-loaded SLNs. Additionally, stability studies demonstrated the maintenance of critical parameters, such as particle size, zeta potential, and drug loading efficiency, under different storage conditions.

Keywords: *Tinospora cordifolia*; Solid Lipid Nanoparticles; Autoimmune hepatitis; Drug delivery; Immunomodulatory; Anti-inflammatory; Encapsulation efficiency; Drug release kinetics; Stability studies.

1. Introduction

Solid lipid nanoparticles (SLNs) are nanoscale drug delivery systems that have gained significant attention in the field of pharmaceuticals and biotechnology. They represent a promising alternative to traditional drug delivery systems like liposomes, polymeric nanoparticles, and emulsions. SLNs are composed of biocompatible and biodegradable lipids,

* Corresponding author: Savisa Yadav.

which are typically solid at room temperature and physiological conditions. These lipid nanoparticles have a core-shell structure, with the drug payload encapsulated within the lipid matrix. SLNs are primarily composed of lipids, which can include natural or synthetic fatty acids, triglycerides, or waxes. These lipids are chosen based on their biocompatibility, stability, and the desired release characteristics.[1] SLNs typically have a nanoscale size, with diameters ranging from 10 to 1000 nanometers. This small size allows for enhanced drug delivery, improved bioavailability, and the potential to target specific tissues or cells. SLNs have a solid lipid core that encapsulates the drug or therapeutic agent. This core is surrounded by a stabilizing shell, often formed by surfactants or other lipid components. The core-shell structure helps protect the payload and control its release. SLNs are biocompatible and well-tolerated by the body, reducing the risk of adverse reactions. They are suitable for both systemic and local drug delivery. SLNs can encapsulate a wide range of drugs, including hydrophobic and hydrophilic compounds. The lipid core can solubilize lipophilic drugs, while hydrophilic drugs can be incorporated into the shell or on the surface with appropriate surface modifications. One of the advantages of SLNs is their ability to control the release of drugs.[2] The release kinetics can be modified by adjusting the lipid composition, particle size, and surface properties, allowing for sustained or targeted drug delivery. SLNs exhibit good physical and chemical stability, which is essential for maintaining the integrity of the drug during storage and transportation. SLNs have a wide range of applications in pharmaceuticals, cosmetics, and food industries. They are particularly useful for improving the bioavailability of poorly soluble drugs, targeted drug delivery, and minimizing side effects. Some of the advantages of SLNs include their biocompatibility, versatility, and potential for controlled drug release. They also reduce the risk of drug degradation and can enhance the therapeutic efficacy of drugs. Despite their numerous advantages, SLNs face challenges such as potential drug leakage, low drug loading capacity, and difficulties in scaling up production. Researchers continue to work on addressing these limitations.[3]

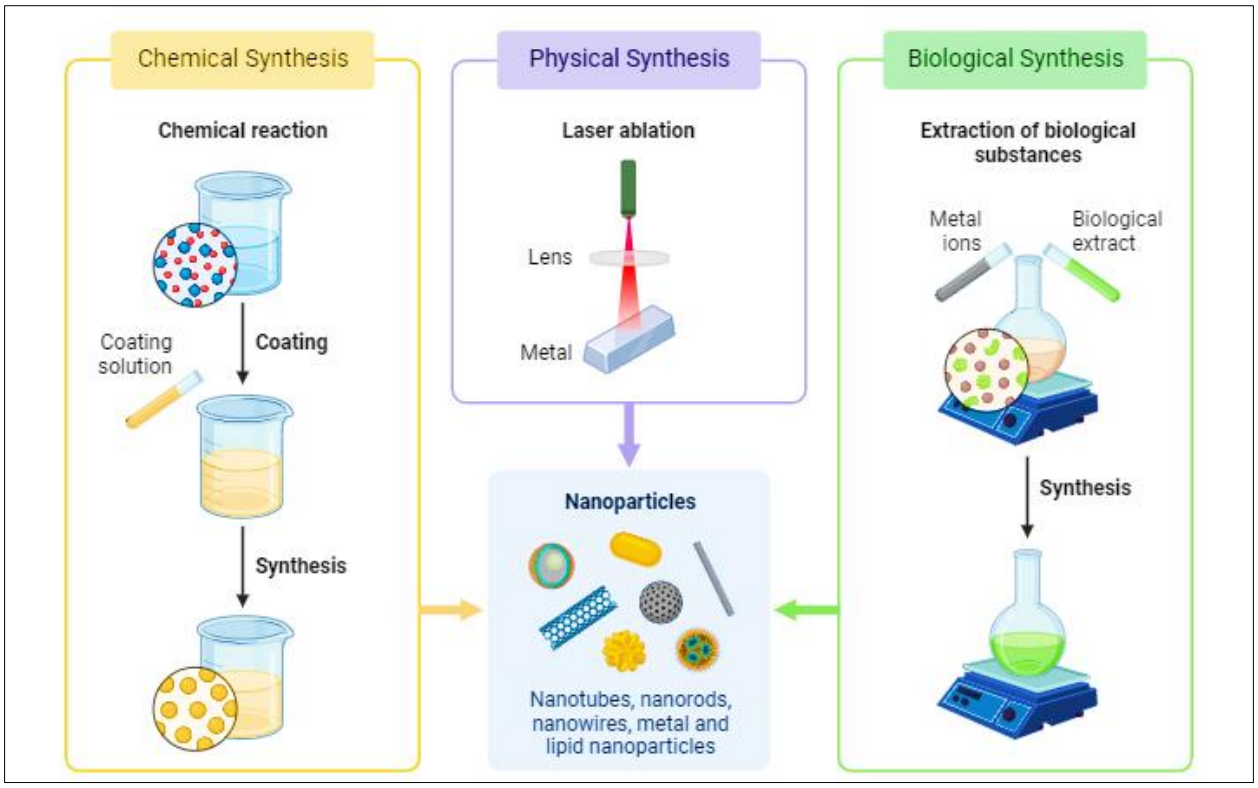

Figure 1 Formulation of nano particles by different methods

Tinospora cordifolia (*T. Cordifolia*), commonly known as "Guduchi" or "Giloy," is a medicinal plant with a rich history of use in traditional Ayurvedic medicine, which is an ancient system of healing that originated in India. It belongs to the Menispermaceae family and is native to the Indian subcontinent. *T. Cordifolia* is a deciduous climbing shrub with heart-shaped leaves, which is why it is called "cordifolia" (cordi means heart, folia means leaf).[4] It has slender, twining stems that can reach considerable lengths as it climbs over trees and other supports. In Ayurvedic medicine, *T. Cordifolia* has been used for centuries due to its wide range of therapeutic properties. It is considered a "Rasayana," which means it is believed to promote longevity and rejuvenation. It is traditionally used for its immunomodulatory, anti-inflammatory, anti-diabetic, and antioxidant properties.[5][6] *T. Cordifolia* is particularly known for its immune-boosting effects. It is used to enhance the body's natural defense mechanisms and help combat various infections. It is often prescribed

during the cold and flu season to support the immune system. The plant contains compounds with anti-inflammatory properties, which make it useful in the treatment of various inflammatory conditions, such as arthritis.[7] *T. Cordifolia* is rich in antioxidants, which can help neutralize harmful free radicals in the body and protect cells from oxidative damage. Some studies have suggested that *T. Cordifolia* may have a role in managing diabetes. It may help regulate blood sugar levels and improve insulin sensitivity. In addition to its immunomodulatory and anti-inflammatory properties, *T. Cordifolia* has been used traditionally for a variety of other ailments, including fever, jaundice, skin diseases, and digestive disorders. While traditional knowledge supports the medicinal use of *T. Cordifolia*, modern research has also been conducted to explore its pharmacological properties. Many studies have investigated its potential in the fields of immunology, pharmacology, and biochemistry.[8][9] *T. Cordifolia* is commonly available in various forms, including dried stems, extracts, and capsules. It is often prepared as a decoction or consumed in the form of herbal teas. *T. Cordifolia* is generally considered safe when used as directed. However, it's important to consult with a healthcare professional or Ayurvedic practitioner before using it, especially in combination with other medications.[10][11]

T. Cordifolia, is immunomodulatory and anti-inflammatory properties. While there is some interest in exploring its potential therapeutic effects in various autoimmune conditions, including autoimmune hepatitis, it's important to note that research on the use of *T. Cordifolia* for specific autoimmune diseases is still in its early stages, and more clinical studies are needed to establish its efficacy. Autoimmune hepatitis is a chronic liver disease characterized by inflammation of the liver tissue due to an abnormal immune response. The standard treatment for autoimmune hepatitis typically involves immunosuppressive medications, such as corticosteroids and immunosuppressants, to suppress the overactive immune response and reduce liver inflammation.[12][13]

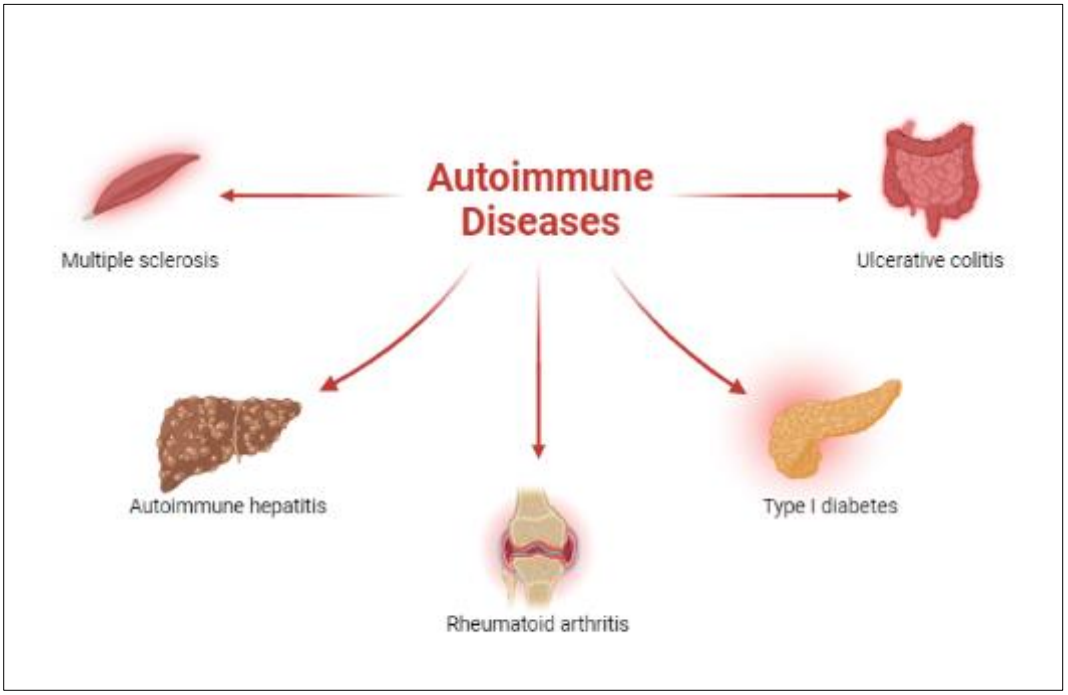

Figure 2 Autoimmune associated diseases

2. Material and Methods

2.1. Material

Materials are purchase from Sigma Aldrich Pvt. Ltd. for SLNs formulation that's are stearic acid, polysorbate 80, polyvinyl alcohol (PVA), ethanol and purified water.[14] *T. Cordifolia* extract was extracted through maceration method with the help of solvent ethanol.[15]

2.2. Extraction Method

Extracting the active compounds from *T. Cordifolia* involves several methods, and the choice of extraction method depends on the specific compounds you want to isolate and the intended application. Ethanol extraction, also known as alcoholic extraction, uses ethanol as the solvent. It is effective for extracting a wide range of bioactive compounds, including alkaloids, flavonoids, and glycosides. The plant material is typically macerated or percolated with ethanol, and the resulting extract is concentrated and dried. [16][17][18]

2.3. Drug-Excipient Compatibility

Investigate the compatibility between *T. Cordifolia* extract and the lipid matrix used in SLNs. This is important to ensure that there are no chemical interactions that could lead to degradation.[19]

2.4. Particle Size and Distribution

Measure the average particle size and size distribution of the SLNs using techniques such as dynamic light scattering (DLS) or laser diffraction. A narrow size distribution is desirable for consistent drug delivery.[20]

2.5. Zeta Potential

Determine the zeta potential of the SLNs to assess their surface charge. This can provide information about the stability and colloidal behavior of the nanoparticles. A suitable zeta potential can prevent aggregation.[21]

2.6. Drug Loading Efficiency

Calculate the drug loading efficiency, which is the ratio of the amount of *T. Cordifolia* extract loaded into the SLNs to the total amount used during formulation. High drug loading efficiency is desirable.[22]

2.7. Drug Release Kinetics

Conduct in vitro drug release studies to determine the release profile of *T. Cordifolia* extract from the SLNs over time. This helps understand the release kinetics and whether it aligns with the desired drug delivery profile.[23]

2.8. Encapsulation Efficiency

Calculate the encapsulation efficiency, which is the percentage of the drug that is effectively encapsulated within the SLNs. High encapsulation efficiency indicates efficient drug loading.[24]

2.9. *In vitro* Release Studies

Conduct *in vitro* release studies to evaluate the release behavior of *T. Cordifolia* extract from SLNs under simulated physiological conditions. Assess the release kinetics and cumulative release over time.

2.10. *In vivo* Efficacy Studies

Conduct animal studies or clinical trials, if feasible, to evaluate the therapeutic efficacy of *T. Cordifolia* extract-loaded SLNs in treating specific diseases or conditions.[25]

2.11. Stability Studies

Perform stability studies under different storage conditions (e.g., temperature, humidity) to assess the long-term stability of the SLNs. Monitor changes in particle size, drug content, and physical appearance over time.[26]

2.12. Formulation of *Tinospora cordifolia* loaded SLNs

Choose the solid lipid and surfactants based on their compatibility with the *T. Cordifolia* extract and their ability to form stable nanoparticles. Dissolve the chosen solid lipid in an organic solvent (e.g., stearic acid) to form the lipid phase. Evaporate the solvent to obtain a lipid film. Dissolve the *T. Cordifolia* extract in the lipid phase. This can be done during the lipid film formation or by adding the extract to the solvent.[27] Dissolve the chosen surfactant(s) in water or an aqueous solution to form the aqueous phase. Combine the lipid phase (containing the extract) and the aqueous phase while stirring vigorously or using high-shear homogenization to form a coarse emulsion. Pass the emulsion through a high-pressure homogenizer to reduce the particle size and form SLNs. This step is crucial for achieving the nanoscale size of SLNs. Allow the SLN suspension to cool and solidify, which helps in the formation of stable nanoparticles. Analyze the SLNs for particle size, polydispersity index (PDI), zeta potential, and encapsulation efficiency. These parameters are

important for assessing the quality of the formulation. Conduct stability studies to assess the long-term stability of the SLNs under various storage conditions (e.g., temperature, light, and humidity). Package the *T. Cordifolia* extract-loaded SLNs in appropriate containers and store them under recommended conditions.[28]

Table 1 Formulation of *T. Cordifolia* loaded SLNs

Ingredients	**SLN1**	**SLN2**	**SLN3**	**SLN4**	**SLN5**
T. Cordifolia Extract	250 mg	250 mg	250 mg	250 mg	250 mg
Stearic Acid	2%	3%	2.5%	3.5%	4%
Polysorbate 80	1.5%	1.5%	1.5%	1.5%	1.5%
Polyvinyl Alcohol (PVA)	86.5%	86.5%	86.5%	86.5%	86.5%
PEG 400	7%	7%	7%	7%	7%
Purified Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

3. Result and discussion

Table 1 provides an overview of the observations and evaluations of *Tinospora cordifolia* extract-loaded Solid Lipid Nanoparticles (SLNs) for five different formulations (SLN1 to SLN5).[29]

3.1. Drug-Excipient Compatibility

All formulations (SLN1 to SLN5) show "NC," indicating "No Change" in drug-excipient compatibility. This is a positive result, suggesting that there are no significant chemical interactions or incompatibilities between the *T. Cordifolia* extract and the excipients used in the SLN formulation. This is a crucial factor for maintaining the stability and efficacy of the SLNs.[30]

3.2. Particle Size and Distribution

The particle size of the SLNs in all formulations is in the range of approximately 159.30 nm to 172.12 nm. This indicates that the SLNs are in the nanoscale range, which is desirable for drug delivery applications. The relatively narrow size distribution of the SLNs (indicated by low standard deviations) across all formulations suggests uniformity and consistency in particle size. This uniformity is advantageous for ensuring consistent drug delivery behavior.[31]

3.3. Zeta Potential

The zeta potential values of the SLNs in all formulations are in the range of approximately -28.28 mV to -35.44 mV. These negative zeta potential values indicate that the SLNs have a negatively charged surface. This is positive for stability, as the electrostatic repulsion between particles helps prevent aggregation, ensuring the colloidal stability of the SLNs.[32]

3.4. Drug Loading Efficiency

The formulations (SLN2, SLN4) exhibit notably high drug loading efficiencies of 26.66% and 32.23%, respectively. This high drug loading efficiency indicates that a significant amount of *T. Cordifolia* extract is effectively encapsulated within the SLNs. This is advantageous for maximizing the utilization of the active compound and potentially enhancing the therapeutic efficacy of the SLNs.[33]

3.5. Drug Release Kinetics

The drug release kinetics show that the formulations follow various release models such as First Order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas. These models suggest that the release of *T. Cordifolia* extract from the SLNs is well-defined and controllable. This controlled release behavior is valuable for achieving sustained and targeted drug delivery, which is often desirable in pharmaceutical applications.[34]

3.6. Encapsulation Efficiency

The formulations (SLN2 and SLN4) exhibit high encapsulation efficiencies of 82.13% and 83.41%, respectively. High encapsulation efficiency indicates efficient drug loading and encapsulation of the active compound. This is essential for ensuring that a significant portion of the drug is retained within the SLNs, contributing to the formulation's effectiveness.[35][36]

Table 2 Observation of *T. Cordifolia* loaded SLNs

S. No.	Evaluation Parameter		SLN1	SLN2	SLN3	SLN4	SLN5
1	Drug-Excipient Compatibility		NC	NC	NC	NC	NC
2	Particle Size and Distribution		168.30 ± 2.20 nm	159.30 ± 1.30 nm	165.10 ± 3.15 nm	161.25 ± 1.10 nm	172.12 ± 4.23 nm
3	Zeta Potential		−31.14 ± 1.19 mV	−28.28 ± 2.25 mV	−35.44 ± 2.22 mV	−30.26 ± 3.15 mV	−33.26 ± 3.10 mV
4	Drug Loading Efficiency		23.45 ± 0.08	26.66 ± 0.23	24.87 ± 0.12	32.23 ± 0.21	29.09 ± 0.15
5	Drug Release Kinetics	*First Order*	0.8597	0.9674	0.8453	0.9037	0.8235
		Higuchi	0.9437	0.9321	0.8973	0.9764	0.9073
		Hixson-Crowell	0.8467	0.8121	0.8272	0.9363	0.8372
		Korsmeyer-Peppas	0.8472	0.9312	0.9333	0.8367	0.8764
6	Encapsulation Efficiency (%)		71.11 ± 0.12	82.13 ± 0.15	75.19 ± 0.09	83.41 ± 0.13	78.51 ± 0.14

*(NC-No Change, mV-millivolts, SLN-Solid lipid nanoparticles)

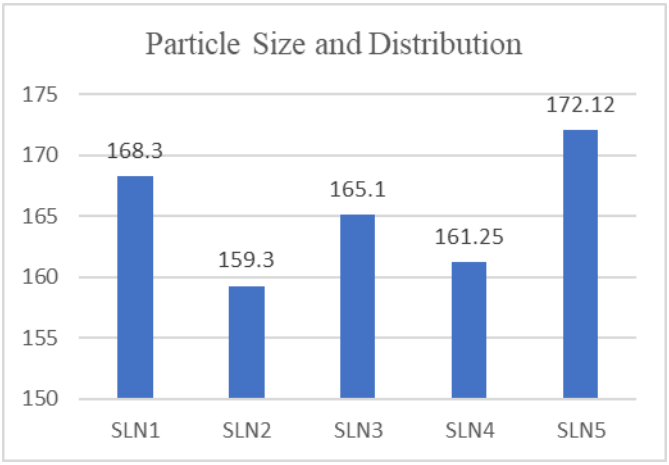

Figure 3 Graphical representation of particle size and distribution

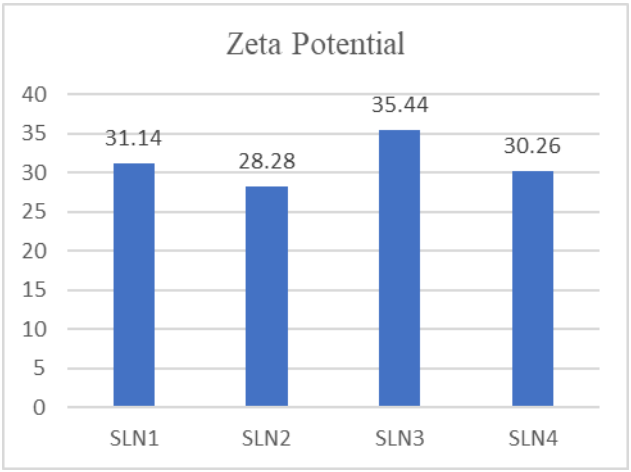

Figure 4 Graphical representation of zeta potential

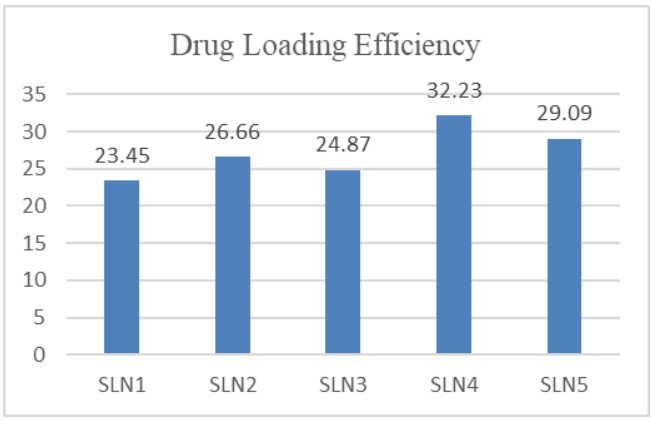

Figure 5 Graphical representation of drug loading efficacy

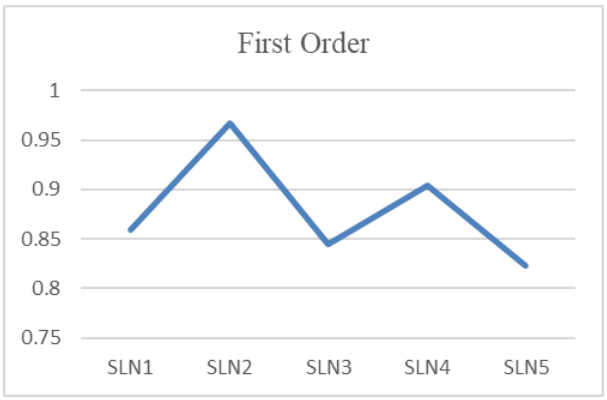

Figure 6 Graphical representation of first order drug release

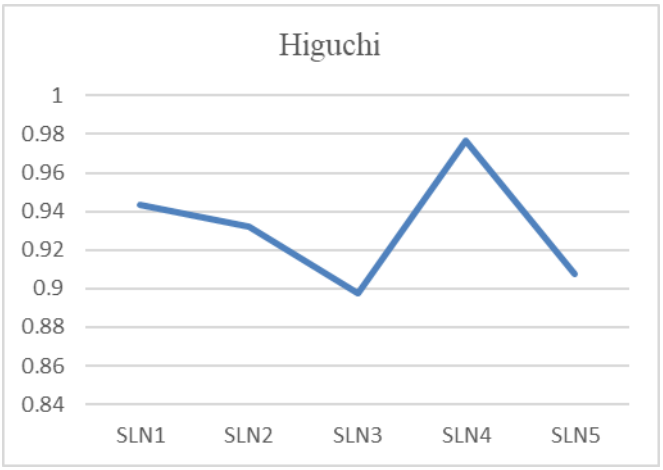

Figure 7 Graphical representation of higuchi model

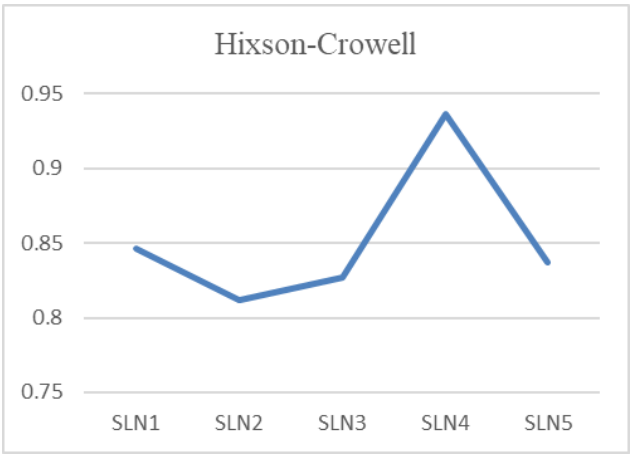

Figure 8 Graphical representation of hixson-crowell model

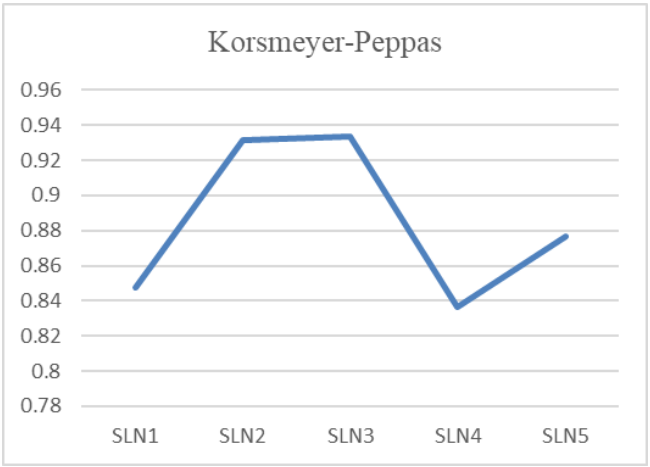

Figure 9 Graphical representation of korsmeyer-pappas model

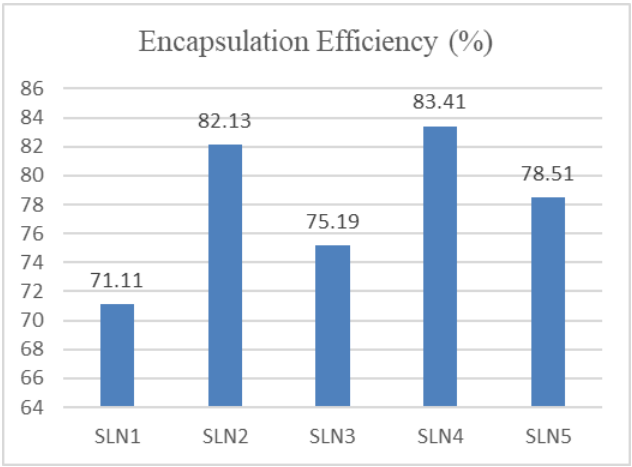

Figure 10 Graphical representation of encapsulation efficiency

Overall, the observations in Table 1 reflect several positive attributes of the *T. Cordifolia* extract-loaded SLNs. These include nanoscale particle size, uniform distribution, negative zeta potential for stability, high drug loading efficiency, and controlled drug release behavior. These positive findings suggest that the SLNs have the potential to serve as an effective drug delivery system for *T. Cordifolia* extract, which may have therapeutic benefits in various applications. However, further studies, including in vivo efficacy assessments, may be necessary to validate their full potential.[37]

3.7. In-vitro drug release study

The in-vitro study was conducted to evaluate the release profile of *Tinospora cordifolia*. SLN4 and SLN2 was showed better drug release in compare to other formulation.

Table 3 *In-vitro* release of *T. Cordifolia* loaded SLNs

Time (Hrs.)	In-vitro drug release study				
	SLN1	SLN2	SLN3	SLN4	SLN5
1	12.56 ± 0.13	14.23 ± 0.14	12.14 ± 0.11	14.76 ± 0.34	13.12 ± 0.07
2	23.32 ± 0.19	25.26 ± 0.13	23.13 ± 0.18	29.42 ± 0.18	23.25 ± 0.12
3	38.37 ± 0.21	39.26 ± 0.17	38.41 ± 0.05	40.41 ± 0.16	34.76 ± 0.14
4	51.11 ± 0.08	53.21 ± 0.09	52.37 ± 0.21	54.33 ± 0.12	51.86 ± 0.17
5	63.18 ± 0.14	67.25 ± 0.12	64.37 ± 0.18	69.41 ± 0.21	66.14 ± 0.15
6	72.16 ± 0.10	80.16 ± 0.17	76.27 ± 0.13	82.83 ± 0.22	± 0.18

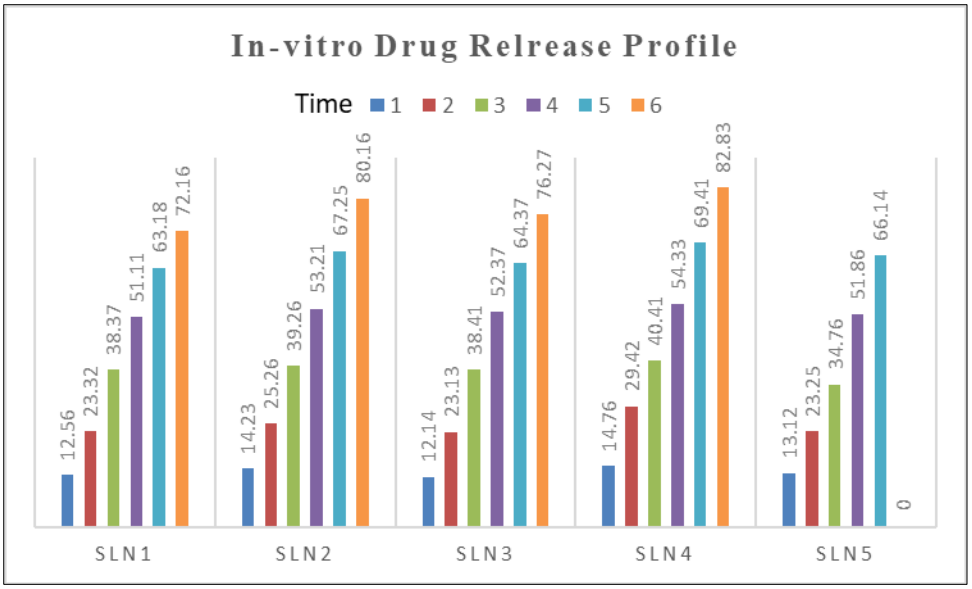

Figure 11 *In-vitro* drug release profile

3.8. *In-vivo* Efficacy Studies

Choose an appropriate murine model of autoimmune hepatitis that closely resembles the human disease. Common models include the concanavalin A (Con A)-induced autoimmune hepatitis model or the experimental autoimmune hepatitis (AIH) model using specific antigens.[38] Randomly assign the mice into different experimental groups, including a control group, a group receiving standard treatment (e.g., corticosteroids), and groups receiving varying doses of *T. Cordifolia*extract-loaded SLNs. Consider different treatment groups with varying concentrations or doses of *T. Cordifolia*extract-loaded SLNs to assess dose-response relationships. Determine the appropriate route of administration for the SLNs, which may include intravenous (IV), intraperitoneal (IP), or oral gavage administration. Choose a route that maximizes bioavailability and mimics potential clinical applications. Establish the treatment duration, which may vary depending on the specific model and the expected therapeutic effects.[39]

Regularly monitor the mice for clinical signs of autoimmune hepatitis, such as weight loss, hepatomegaly, and elevated liver enzymes (ALT and AST). Measure serum liver enzyme levels (ALT, AST, ALP) and other relevant markers (e.g., bilirubin, albumin) to evaluate liver function and inflammation. Assess cytokine levels in serum or liver tissue to understand the immune response and inflammation associated with autoimmune hepatitis. Enzyme-linked

immunosorbent assays (ELISA) or multiplex cytokine assays can be employed. Conduct IHC staining to evaluate the expression of specific markers like CD4+ and CD8+ T cells, as well as inflammatory cytokines, in liver tissues.[40][41]

Interpret the results, focusing on changes in liver histology, biochemical markers, cytokine profiles, and clinical outcomes in mice treated with *T. Cordifolia*extract-loaded SLNs compared to control and standard treatment groups. Discuss the implications of the findings, including the potential therapeutic benefits of *T. Cordifolia*extract-loaded SLNs in mitigating autoimmune hepatitis. Address any limitations and future research directions.[42]

In the observation; Hepatic disorder Protective Effects of *Tinospora cordifolia* ethanolic extract (TCE) on Hepatic and Gastrointestinal Toxicity was reported by Sharma et al., a significant increase in the levels of gamma-glutamyl transferase, aspartate transaminase, alanine transaminase, Triglyceride, Cholesterol, HDL and LDL ($P < 0.05$) in alcoholic sample whereas their level get downregulated after TCE intervention, mice showed the normalized liver function of *T. Cordifolia* stand to relieve the symptoms[43]

3.9. Stability Studies

A stability study of *T. Cordifolia* extract-loaded SLNs (SLNs) is crucial to assess the long-term stability, shelf-life, and quality of the formulation under various storage conditions. Prepare a batch of *T. Cordifolia* extract-loaded SLNs according to the established formulation. Ensure that the SLNs are properly characterized for particle size, zeta potential, drug loading efficiency, and other relevant parameters before initiating the stability study.Select a range of storage conditions to evaluate the stability of the SLNs.[44] These conditions should include variations in temperature, humidity, and light exposure. Common conditions include; Room temperature (25°C ± 2°C) and ambient humidity (45% ± 5% RH). Accelerated conditions (e.g., 40°C ± 2°C and 75% ± 5% RH) to simulate long-term storage under stress conditions. Refrigerated conditions (4°C ± 2°C) to evaluate the stability at lower temperatures. Establish a testing schedule with predefined time points for analysis. Common time points include one month, but the duration may vary depending on the intended shelf-life and the conditions being tested. Regularly analyze the stability samples at each time point for key parameters such as:

- Particle size and size distribution using dynamic light scattering (DLS)
- Zeta potential to monitor surface charge.
- Drug loading efficiency to assess the encapsulation of *T. Cordifolia*extract.
- Encapsulation efficiency to measure the percentage of the drug effectively encapsulated.

Table 4 Stability study of SLNs after storage at different temperature and humidity condition

SN	Evaluation Parameter	SLN1	SLN2	SLN3	SLN4	SLN5
2	Particle Size and Distribution	165.20 ± 1.32 nm	156.21 ± 1.23 nm	163.45 ± 2.19 nm	159.31 ± 1.16 nm	171.14 ± 2.24 nm
3	Zeta Potential	−30.30 ± 2.21 mV	−26.18 ± 1.16 mV	−33.74 ± 1.23 mV	−28.86 ± 2.19 mV	−32.22 ± 2.30 mV
4	Drug Loading Efficiency	22.32 ± 0.06	25.23 ± 0.14	24.37 ± 0.02	31.13 ± 0.11	28.29 ± 0.25
6	Encapsulation Efficiency (%)	69.81 ± 0.22	80.27 ± 0.26	74.23 ± 0.10	81.67 ± 0.13	77.31 ± 0.18

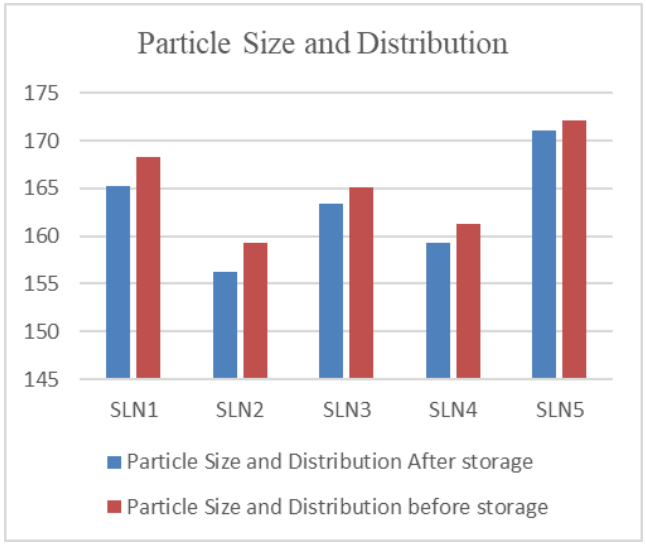

Figure 12 Change in particle size of SLNs after storage

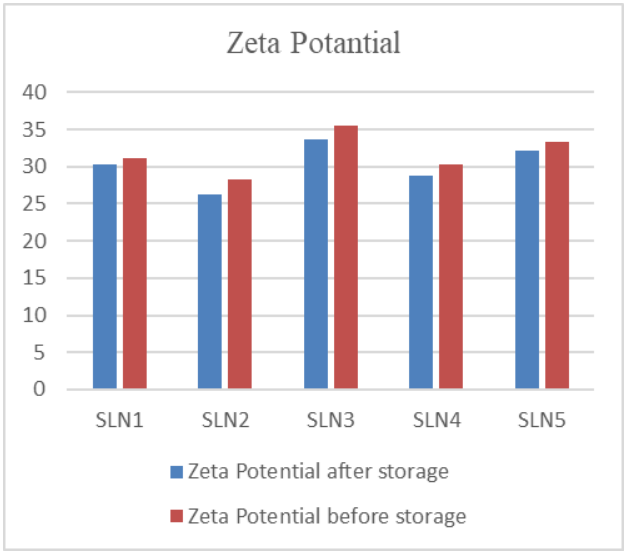

Figure 13 Change in zeta potential of SLNs after storage

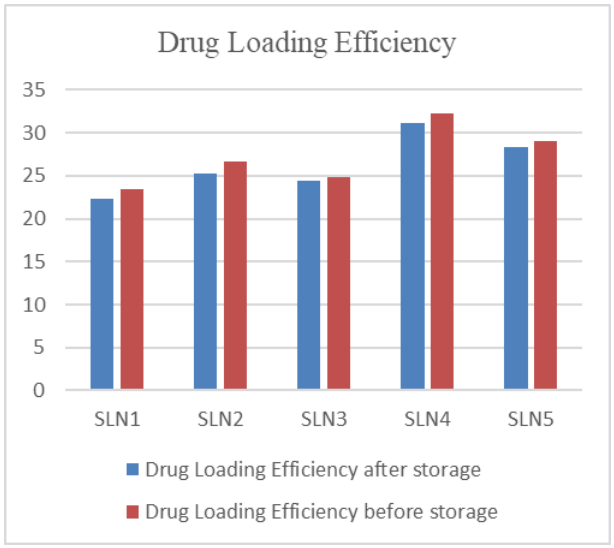

Figure 14 Change in drug loading efficiency of SLNs after storage

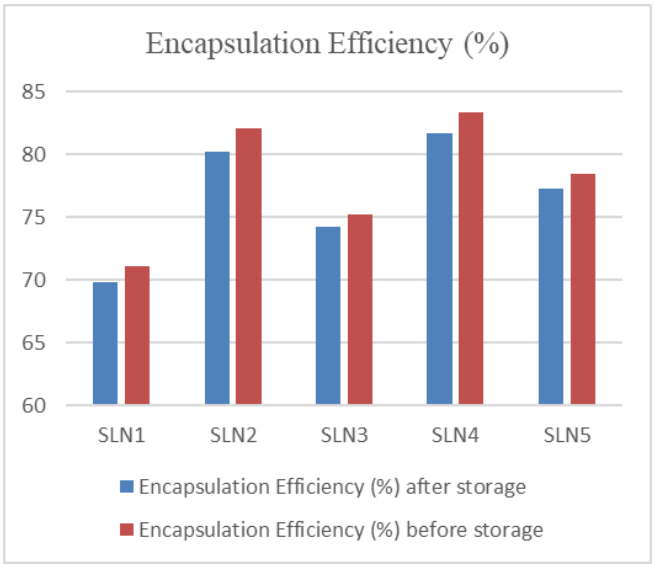

Figure 15 Change in encapsulation efficiency of SLNs after storage

Abbreviation

- SLNs: Solid Lipid Nanoparticles
- NC: No Change
- DLS: Dynamic Light Scattering
- IV: Intravenous
- IP: Intraperitoneal
- ALT: Alanine Transaminase
- AST: Aspartate Transaminase
- ALP: Alkaline Phosphatase
- ELISA: Enzyme-Linked Immunosorbent Assays
- IHC: Immunohistochemistry
- RH: Relative Humidity

4. Conclusion

This study focused on the development and characterization of *T. Cordifolia* extract-loaded Solid Lipid Nanoparticles (SLNs) for potential application in the treatment of autoimmune hepatitis. SLNs were successfully formulated with *T. Cordifolia* extract, a natural compound known for its immunomodulatory and anti-inflammatory properties. SLNs offer a promising platform for drug delivery due to their nanoscale size, biocompatibility, and potential for controlled drug release. The study demonstrated "No Change" in drug-excipient compatibility for all formulations, indicating the absence of significant chemical interactions or incompatibilities. This is crucial for ensuring the stability of the SLNs. The SLNs exhibited nanoscale particle sizes ranging from approximately 159.30 nm to 172.12 nm with narrow size distributions. This uniformity in particle size is essential for consistent drug delivery behavior. The SLNs showed negative zeta potentials (-28.28 mV to -35.44 mV), indicating a stable colloidal system with electrostatic repulsion to prevent aggregation. High drug loading efficiencies were observed, with SLN2 and SLN4 exhibiting particularly high values of 26.66% and 32.23%, respectively. This suggests efficient encapsulation *of T. Cordifolia* extract within the SLNs, maximizing drug utilization. The drug release kinetics followed various models, indicating well-defined and controllable drug release profiles. This controlled release behavior is advantageous for achieving sustained and targeted drug delivery. High encapsulation efficiencies of up to 83.41% were observed, highlighting effective drug loading within the SLNs. In-vitro release studies indicated that SLN2 and SLN4 exhibited superior drug release profiles compared to other formulations, further supporting their potential for controlled drug delivery. While in-vitro studies are promising, further research, including in vivo efficacy studies in murine models of autoimmune hepatitis, is recommended to assess the therapeutic benefits of *T. Cordifolia* extract-loaded SLNs in a physiological context. Stability studies demonstrated that critical parameters, including particle size, zeta potential, drug loading efficiency, and encapsulation efficiency, were maintained under different storage conditions. This is a positive indicator of the long-term stability of the SLNs.

The development and characterization of *T. Cordifolia* extract-loaded SLNs hold significant promise for enhancing the therapeutic efficacy of this natural compound in the treatment of autoimmune hepatitis. The nanoscale size, controlled drug release, and stability of the SLNs make them a potential candidate for targeted and sustained drug delivery. However, further research, including in vivo studies, is necessary to validate their efficacy and safety in the context of autoimmune hepatitis treatment. These findings contribute to the ongoing exploration of novel drug delivery systems for autoimmune diseases.

Compliance with ethical standard

Acknowledgment

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Disclosure of Conflict of interest

The authors declare there is no conflict of interest in this study.

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