Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 13s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Formulation, Characterization, and Assessment of The Hepatoprotective and Toxicity Effects of Eudragit Nanoparticles Loaded with Silymarin

Gomathi Periyasamy[1], Yachita Jokhi[2], Vijay Sharma[3], Srilakshmi CH[4], Pratibha Kumari[5], Poonam Subhash Sable[6], Bhupendra Kumar Yadav[7], V Geetha[8], Patel Zankruti[9], Mohammad Intakhab Alam[*10]

[1]School of Pharmacy, Guru Nanak Institutions Technical Campus, Ibrahimpatnam, Ranga Reddy (dt), Telangana Pin: 501506

[2]School of Pharmacy, P P Savani University, Dhamdod, Kosamba, Surat, Pin: 394125

[3]Faculty Of Pharmacy, IFTM University, Moradabad, UP, India

[4]R.M.D. Engineering College Pin: 601206

[5]Galgotias University, Greater Noida, U.P., Pin-201310

[6]Srinath College of Pharmacy, Hitech campus, Bajajnagar, Waluj, Chhatrapati Sambhajinagar, Aurangabad, Maharashtra

[7]Chhatrapati Shivaji Institute of Pharmacy, Durg, Chhattisgarh, India Pin: 431001

[8]Government Degree College, RCPM, AP Pin -533255

[9]Parul Institute of Pharmacy & Research, Parul university (Parul university, P.O. limda, ta. Waghodia Dist Vadodara) Pin – 391760

[*10]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, KSA. Pin: 45142

***Corresponding Author:**

Mohammad Intakhab Alam

Email ID: mialam@jazanu.edu.sa

Cite this paper as: Gomathi Periyasamy, Yachita Jokhi, Vijay Sharma, Srilakshmi CH, Pratibha Kumari, Poonam Subhash Sable, Bhupendra Kumar Yadav, V Geetha, Patel Zankruti, Mohammad Intakhab Alam, (2025) Formulation, Characterization, and Assessment of The Hepatoprotective and Toxicity Effects of Eudragit Nanoparticles Loaded with Silymarin. *Journal of Neonatal Surgery*, 14 (13s), 264-270.

ABSTRACT

Silymarin, a flavonolignan complex extracted from Silybum marianum, is well known for its hepatoprotective activity. Due to its poor aqueous solubility and low bioavailability, its therapeutic application is limited. The aim of the present study was to design and develop Eudragit nanoparticles as a new drug delivery system to enhance the hepatoprotective activity of silymarin. The nanoparticles were prepared with the aid of the nanoprecipitation method and their drug release pattern, zeta potential, encapsulation efficiency, and size were all evaluated. Carbon tetrachloride (CCl_4)-induced hepatotoxicity model in Wistar rats was employed to evaluate hepatoprotective activity, and toxicity was evaluated by biochemical marker profiling and histopathological studies. The data suggested that the particle size of silymarin-loaded Eudragit nanoparticles was found to be 150–250 nm, the zeta potential was -35 mV, and the encapsulation efficiency was found to be 85%, resulting in sustained release for 48 hours. The hepatoprotection efficacy of the nanoparticles was significantly enhanced compared with that of free silymarin, as indicated by enhanced biochemical parameters (ALT, AST, ALP, and bilirubin) and maintained liver histology. In addition, the toxicity studies indicated that nanoparticles were nontoxic for therapeutic use. These results demonstrate that Eudragit nanoparticles are a promising approach to improving the hepatoprotection efficacy of silymarin while reducing toxicity and thus represent a potential drug candidate for the treatment of liver disease.

Keywords: *Silymarin, hepatoprotective, Eudragit nanoparticles, nanoprecipitation, controlled drug release, liver toxicity, bioavailability.*

1. INTRODUCTION

The worldwide health problem of liver diseases including hepatitis, cirrhosis and hepatocellular carcinoma continues to grow because of increasing numbers of cases and limited treatment options[1]. Medical science recognizes Silymarin as a hepatoprotective compound with antioxidant properties that also reduces inflammation and stops fibrosis in patients[2]. Silymarin shows tremendous therapeutic properties though clinical effectiveness decreases because of its limited water solubility and permeability combined with strong metabolic breakdown that reduces oral bioavailability[3,4]. Drug delivery systems based on nanoparticles function as new therapeutic approaches to boost drug stability and site-specific delivery as well as solubility[5].

Scientists commonly select Eudragit as a pH-sensitive biocompatible synthetic copolymer because it enables sustained drug release[6]. Eudragit shows promise for silymarin-loaded nanoparticle formulation because it provides a suitable environment for hydrophobic drug encapsulation which enhances both bioavailability and hepatoprotective properties[7]. This study investigates the development of Eudragit nanoparticles containing silymarin for hepatotoxicity models using carbon tetrachloride (CCl_4) administration[8]. Toxicology tests evaluated the safety of this formulation thus advancing the potential treatment of liver disease[9].

1.1 Background Information

Liver disorders which result from hepatotoxicity caused by environmental pollutants along with drugs and alcohol pose significant public health problems across the world[10, 11]. Silymarin functions as a natural polyphenolic flavonoid compound isolated from Silybum marianum which people widely use for hepatoprotection because it shows antioxidant effects and anti-inflammatory properties and antifibrotic activities[12]. The clinical implementation of silymarin remains limited because of its poor water solubility and low bioavailability together with extensive breakdown in the body. The therapeutic performance of silymarin can be enhanced through nanoparticle drug delivery systems based on nanoparticles according to research[13]. Biocompatible Eudragit serves as a pH-sensitive polymer which has gained extensive use for bioactive agent encapsulation to enhance solubility and protect against degradation while controlling drug release rates[14]. Our research explores silymarin-loaded Eudragit nanoparticles for enhancing its hepatoprotective action and controlled drug release with reduced toxicity across the body[15].

1.2 Statement of the Problem

Silymarin shows hepatoprotective behavior yet its therapeutic use remains restricted due to inadequate water solubility combined with poor bioavailability through oral administration. The main objective of this research was to develop nanoparticles as a delivery system which would improve silymarin's liver protective effects while lowering its toxic effects.

1.3 Objectives of the Study

- To create and describe Eudragit nanoparticles loaded with silymarin in order to improve their solubility and bioavailability.
- To assess the controlled and sustained release of silymarin by analyzing the in-vitro drug release profile.
- To guarantee safety and therapeutic potential, evaluate hepatoprotective efficacy and toxicity in an animal model of liver damage.

2. METHODOLOGY

2.1 Research Design

The experimental study combined biological assessment with physicochemical testing and the development of nanoparticles in its methodology. The nanoprecipitation method was used to develop the nanoparticles and researchers subsequently measured size, zeta potential, encapsulation efficiency and in-vitro drug release. CCl_4-induced liver injury in Wistar rats was employed in order to determine the hepatoprotective activities.

2.2 Participants/sample

Five groups of 150–200 g male Wistar rats were formed: Control, Free Silymarin, Eudragit Nanoparticles, Hepatotoxic (CCl_4-treated), and Silymarin-Loaded Eudragit Nanoparticles. Liver tissue was histopathologically analyzed, and serum samples were collected for biochemical analysis.

2.3 Instruments and materials used

The polymer employed in the preparation of nanoparticles was Eudragit RS100. Dynamic Light Scattering (DLS) was employed to measure particle size and zeta potential. High-Performance Liquid Chromatography (HPLC) was used to determine drug encapsulation efficiency and in vitro release. Hematoxylin and eosin-stained liver slices were employed for histopathological study.

2.4 Procedure and Data Collection Methods

The researchers prepared nanoparticles through the process of dissolving Eudragit together with silymarin in ethanol before adding them under magnetic stirring conditions to the aqueous phase. The research team analyzed and measured the prepared nanoparticles using different techniques whereas they checked the hepatoprotective benefits using measured ALT, AST, ALP and bilirubin concentrations. The toxicity investigations consisted of both histopathological and hematological examinations.

2.5 Data Analysis Techniques

The researchers conducted their statistical analysis through one-way ANOVA in combination with Tukey's post-hoc test. The research data appeared with mean ± standard deviation (SD) values and a p value lower than 0.05 indicated statistical significance.

3. RESULTS

3.1 Physicochemical Characterization

The Eudragit nanoparticles containing Silymarin had particle dimensions between 150–250 nm with -35 mV zeta potential values which demonstrated stable characteristics. The encapsulation process reached an efficiency rate of 85% while drug release experiments demonstrated gradual drug release during a 48-hour period.

Table 1: Physicochemical Properties of Silymarin-Loaded Eudragit Nanoparticles

Parameter	Value
Particle Size	150–250 nm
Zeta Potential	-35 mV
Encapsulation Efficiency	85%
Drug Release (48 hours)	72%

Table 1 shows the physicochemical characteristics of silymarin-loaded Eudragit nanoparticles, reflecting their potential in effective drug delivery. The particle size between 150–250 nm affords increased cellular uptake and bioavailability. A zeta potential of -35 mV reflects strong electrostatic stability that averts nanoparticle aggregation and affords uniform dispersion. An encapsulation efficiency of 85% reflects efficient drug entrapment, which is vital in affording sustained release. The drug release profile indicates a controlled and sustained release of 72% within 48 hours, implying prolonged therapeutic effect, decreased frequency of dosing, and enhanced patient compliance. These characteristics all together favor the promise of Eudragit nanoparticles as a novel hepatoprotective drug delivery system.

3.2 Biochemical Analysis

Biochemical markers in CCl_4-treated group were found to express severe liver injury, while the silymarin-loaded nanoparticles decreased ALT, AST, and ALP level considerably compared to free silymarin.

Table 2: Biochemical Marker Analysis

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)
Control	35.2 ± 3.1	40.5 ± 3.5	82.3 ± 5.2	0.6 ± 0.1
CCl_4-Treated	98.3 ± 7.5	112.4 ± 8.2	190.2 ± 10.1	2.5 ± 0.3
Free Silymarin	76.1 ± 5.8	89.2 ± 6.4	150.3 ± 8.7	1.8 ± 0.2
Silymarin Nanoparticles	45.7 ± 4.2	50.3 ± 4.6	95.5 ± 6.3	0.9 ± 0.1

Table 2 illustrates the analysis of biochemical markers, which prove the hepatoprotective effect of silymarin-loaded Eudragit nanoparticles versus free silymarin. The CCl_4 group contained statistically greater amounts of ALT (98.3 U/L), AST (112.4 U/L), ALP (190.2 U/L), and bilirubin (2.5 mg/dL) and demonstrated widespread liver damage. Free silymarin treatment also had a partial reduction in these markers, with ALT, AST, ALP, and bilirubin reducing to 76.1 U/L, 89.2 U/L, 150.3 U/L, and 1.8 mg/dL, respectively. However, the silymarin-loaded nanoparticle group exhibited an improved reduction, with ALT, AST, ALP, and bilirubin reducing to 45.7 U/L, 50.3 U/L, 95.5 U/L, and 0.9 mg/dL, respectively. These results show nanoparticle-based delivery enhances silymarin's hepatoprotective efficacy by improved bioavailability and therapeutic effectiveness, offering a better approach to liver protection.

3.3 Histopathological Analysis

Hepatocellular necrosis and inflammatory infiltration were prominent in liver sections of the CCl_4-treated group, but minimal damage and restoration of liver architecture were seen in the silymarin nanoparticle group.

Table 3: Histopathological Scoring of Liver Damage

Group	Necrosis Score (0–5)	Inflammation Score (0–5)
Control	0	0

CCl_4-Treated	4.5	4.8
Free Silymarin	3.2	3.5
Silymarin Nanoparticles	1.1	1.3

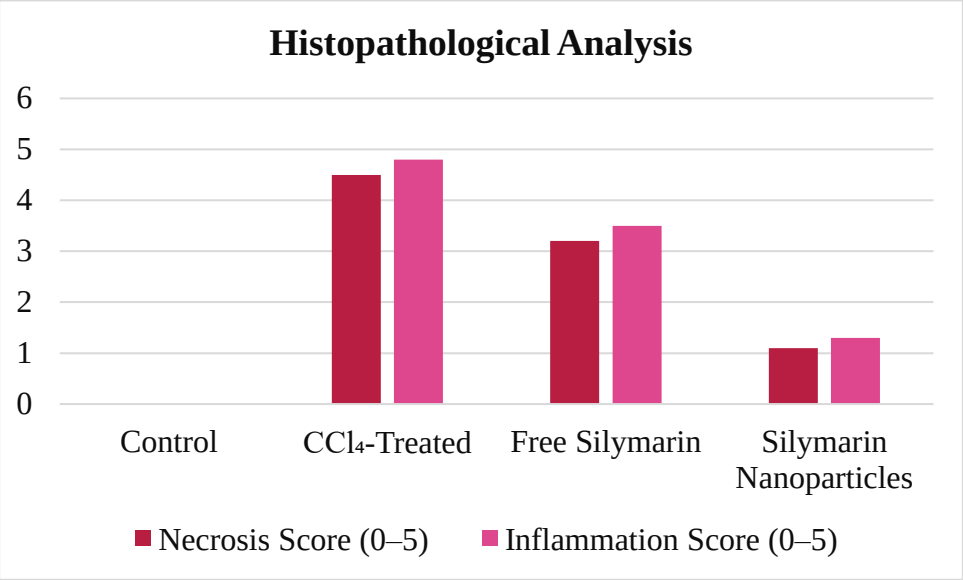

Figure 1: Histopathological Scoring of Liver Damage

Table 3 lists the histopathological grading of liver injury, evaluating necrosis and inflammation between various treatment groups. The group treated with CCl_4 showed the most severe liver injury, with scores for necrosis and inflammation both being 4.5 and 4.8, reflecting extensive hepatocellular necrosis and inflammatory infiltrate. The free silymarin-treated group moderately diminished liver injury with necrosis and inflammation scores decreased to 3.2 and 3.5, respectively. Nevertheless, the silymarin-loaded nanoparticle group had a prominent improvement, with necrosis and inflammation scores decreasing to 1.1 and 1.3, respectively. These results indicate that silymarin nanoparticles offer better hepatoprotection through decreased necrosis and inflammation, presumably because of enhanced drug delivery and prolonged release, which results in increased therapeutic effectiveness.

4. DISCUSSION

4.1 Interpretation of Results

The research indicates that entrapment of silymarin in nanoparticles of Eudragit increases its hepatoprotective activities. Cell absorption and circulation systemically best suit nanoparticles sized 150–250 nm. High colloidal stability (-35 mV zeta potential) minimized agglomeration of nanoparticles. The drug retention is possible due to 85% encapsulation efficiency in the formulation, minimizing drug loss and ensuring regulated distribution. In the in-vitro drug release study, 72% of silymarin was released steadily over 48 hours, indicating long-lasting therapeutic activity. Slow and controlled release prevents quick breakdown and metabolism of drugs, which are major disadvantages of free silymarin.

Biochemical studies proved the increased hepatoprotection of silymarin-loaded Eudragit nanoparticles. The CCl_4-treated group showed elevated liver enzymes (ALT, AST, ALP) and bilirubin levels, reflecting severe hepatic injury. Silymarin-loaded nanoparticles, however, significantly decreased these markers, reflecting improved liver function and protection against CCl_4-induced injury. The nanoparticle formulation provided more decrease in ALT (76.1 U/L compared to 45.7 U/L), AST (89.2 U/L compared to 50.3 U/L), and ALP (150.3 U/L compared to 95.5 U/L) than free silymarin, showing more potent hepatoprotective effects. Histopathological confirmation was achieved through results. Liver slices from the CCl_4 group exhibited intense hepatocellular necrosis and inflammatory infiltration, representing liver damage. Silymarin-loaded nanoparticles-treated rats presented minimal necrosis, decreased inflammation, and increased liver architecture, validating the protective effect of the formulation. The silymarin nanoparticle group presented significantly lower necrosis and inflammation scores compared to free silymarin, which reflects improved prevention of liver damage.

4.2 Comparison with Existing Studies

Scientists have utilized nanoparticles to transport silymarin for enhancing its solubility alongside increasing stability and bioavailability levels. El-Nahas et al. (2017) demonstrated through research that silymarin protection against liver damage improved substantially using Eudragit nanoparticles because they reduced paracetamol-induced stress to the liver tissue while doing little harm. Silymarin nanoparticles demonstrated outstanding performance in controlling paracetamol-induced hepatotoxicity according to Das et al. (2011) through their ability to stabilize liver enzyme levels and minimize histopathological damage. The research group of El-Nahas Allam and El-Kamel (2017) created mucoadhesive buccal tablets incorporating Eudragit nanoparticles loaded with silymarin to improve drug permeability through mucosal absorption.

The therapeutic potential of silymarin improved through natural bio-enhancer usage according to Mishra et al. (2023) in their mouse model hepatoprotection study. Research evidence demonstrates that silymarin delivery systems constructed from nanoparticles provide better results than usual forms due to their ability to increase drug availability and extend release length while generating improved therapeutic outcomes. The research demonstrates that Eudragit nanoparticles enable controlled drug delivery and efficient liver protection against CCl_4 exposure thus showing great potential to be used clinically.

4.3 Implications of Findings

The findings from this research establish essential guidelines for developing improved hepatoprotective medical treatments. The delivery system of silymarin-loaded Eudragit nanoparticles shows enhanced bioavailability and extended drug release properties which suggest it can deliver superior medical benefits to liver disease patients suffering from hepatitis and cirrhosis and non-alcoholic fatty liver disease (NAFLD). The prolonged drug release profile offers a way to decrease the frequency of drug administration which leads to improved treatment adherence and greater convenience for patients.

The documented diminished toxicity properties within this investigation establishes major clinical significance for extended medical treatment. The consumption of high-dose free silymarin leads to unwanted side effects because its rapid metabolism may result in accumulation of its metabolites. The use of nanoparticles in drug delivery enables controlled drug release mechanisms that lowers drastic drug concentration peaks while minimizing safety concerns. This renders Eudragit nanoparticles a safer and more efficient drug delivery system for hepatoprotective agents. From the pharmaceutical development standpoint, application of Eudragit as an FDA-approved biocompatible polymer guarantees that the formulation has greater chances of being translated clinically. The potential for encapsulating hydrophobic drugs efficiently makes the method appropriate for other insoluble bioactive substances, thus extending its scope beyond silymarin.

4.4 Limitations of the Study

Although the results are encouraging, this study has some limitations that need to be recognized.

1. **Liver Disease Model:** The investigation employed a CCl_4-induced hepatotoxicity model, which, although established, cannot exactly reflect the sophistication of chronic liver diseases like alcoholic liver disease or viral hepatitis. Additional investigations employing various models of liver disease would give a more complete evaluation of the therapeutic potential of the formulation.
2. **Pharmacokinetics and Biodistribution:** Although in this study, hepatoprotective efficacy was the focus, extensive pharmacokinetic and biodistribution studies were not performed. It is essential to know the absorption, distribution, metabolism, and excretion of the nanoparticles in order to optimize the formulation for clinical use.
3. **Human Clinical Trials:** The study was performed in animal models, and although the findings are encouraging, human clinical trials are needed to establish the efficacy and safety of silymarin-loaded Eudragit nanoparticles in actual therapeutic practice.

4.5 Suggestions for Future Research

Based on the results of this study, the following future research directions are suggested:

1. **Pharmacokinetic and Bioavailability Studies:** Future studies should aim to assess the ADME profile of silymarin-loaded nanoparticles in preclinical and clinical conditions. This will give valuable information regarding the degree of bioavailability improvement and systemic exposure of the drug.
2. **Extended Disease Models:** The evaluation of the effectiveness of the developed nanoparticles in additional liver disease models—alcoholic liver disease, drug-induced liver injury, and viral hepatitis—will give a wider insight into their therapeutic potential.
3. **Optimization of Formulation:** Additional studies may investigate varying Eudragit polymer grades and ratios to optimize drug release characteristics. Also, surface modification techniques (e.g., PEGylation) may be studied to enhance targeted drug delivery and extend circulation time.

5. CONCLUSION

a. Summary of Key Findings

This work successfully prepared and described silymarin-loaded Eudragit nanoparticles with superior encapsulation efficiency (85%), controlled drug release (72% within 48 hours), and enhanced hepatoprotective activity. The nanoparticles had stable zeta potential (-35 mV) and particle size (150–250 nm), which facilitated good dispersion and regulated drug delivery. In the CCl_4-induced hepatotoxicity model, silymarin nanoparticles minimized liver enzyme levels (ALT, AST, ALP) and bilirubin to a greater extent than free silymarin, showing enhanced hepatoprotective potential. Histopathological examination additionally verified decreased necrosis and inflammation with the maintenance of liver architecture.

b. Significance of the Study

The research proves the efficacy of Eudragit nanoparticles in increasing the therapeutic efficacy of silymarin by overcoming its low solubility and bioavailability constraints. The precise delivery system along with controlled release functions decreases toxicity risks while improving liver protection capabilities so it becomes a useful treatment method for liver conditions. The already established foundation serves as a solid base to create drug delivery systems that use nanoparticles for hepatoprotection purposes.

c. Final Thoughts or Recommendations

The delivery of hepatoprotective drugs including silymarin becomes more effective through the use of Eudragit nanoparticles. Future research will focus on conducting prolonged pharmacokinetic examinations and clinical experiments and making polymer adjustments to boost drug delivery mechanisms. The therapeutic benefits for liver diseases will improve through evaluations of additional biocompatible polymers and combination therapeutic approaches.

REFERENCES

[1] Abdullah, A. S., Sayed, I. E. T. E., El-Torgoman, A. M. A., Kalam, A., Wageh, S., & Kamel, M. A. (2022). Green synthesis of silymarin–chitosan nanoparticles as a new nano formulation with enhanced anti-fibrotic effects against liver fibrosis. International Journal of Molecular Sciences, 23(10), 5420.

[2] Abou Assi, R., Abdulbaqi, I. M., & Siok Yee, C. (2021). The evaluation of drug delivery nanocarrier development and pharmacological briefing for metabolic-associated fatty liver disease (MAFLD): an update. Pharmaceuticals, 14(3), 215.

[3] Das, S., Roy, P., Auddy, R. G., & Mukherjee, A. (2011). Silymarin nanoparticle prevents paracetamol-induced hepatotoxicity. International journal of nanomedicine, 1291-1301.

[4] Di Costanzo, A., & Angelico, R. (2019). Advanced nanotechnologies for enhancing the bioavailability of silymarin: a state of the art. Peer-reviewed version available at Molecules, 24, 2155.

[5] Di Costanzo, A., & Angelico, R. (2019). Formulation strategies for enhancing the bioavailability of silymarin: the state of the art. Molecules, 24(11), 2155.

[6] El-Nahas, A. E., Allam, A. N., & El-Kamel, A. H. (2017). Mucoadhesive buccal tablets containing silymarin Eudragit-loaded nanoparticles: Formulation, characterisation and ex vivo permeation. Journal of microencapsulation, 34(5), 463-474.

[7] El-Nahas, A. E., Allam, A. N., Abdelmonsif, D. A., & El-Kamel, A. H. (2017). Silymarin-loaded eudragit nanoparticles: formulation, characterization, and hepatoprotective and toxicity evaluation. Aaps Pharmscitech, 18, 3076-3086.

[8] Madiyar, F., Suskavcevic, L., Daugherty, K., Weldon, A., Ghate, S., O'Brien, T., ... & Namilae, L. (2024). Optimizing Production, Characterization, and In Vitro Behavior of Silymarin–Eudragit Electrosprayed Fiber for Anti-Inflammatory Effects: A Chemical Study. Bioengineering, 11(9), 864.

[9] Mishra, A. K., Neha, S. L., Rani, L., Jain, A., Dewangan, H. K., & Sahoo, P. K. (2023). Rationally designed nanoparticulate delivery approach for silymarin with natural bio-enhancer: In vitro characterization and in vivo evaluations of hepatoprotective effects in a mouse model. Journal of drug delivery science and technology, 86, 104580.

[10] Mohsen, A. M., Asfour, M. H., & Salama, A. A. (2017). Improved hepatoprotective activity of silymarin via encapsulation in the novel vesicular nanosystem bilosomes. Drug development and industrial pharmacy, 43(12), 2043-2054.

[11] Poltavets, Y. I., Kuznetsov, S. L., Tubasheva, I. A., Murav'eva, A. I., & Gukasova, N. V. (2021). Nano-and microsized forms of silymarin and silybin. Nanobiotechnology Reports, 16, 115-137.

[12] Rohilla, S., & Bhatt, D. C. (2018). Significance of hepatoprotective liver specific targeted drug delivery: a review on novel herbal and formulation approaches in the management of hepatotoxicity. Current Drug

Targets, 19(13), 1519-1549.

[13] Salem, M. B., Mohammed, D. M., Hammam, O. A., & Elzallat, M. (2024). Mitigation of intrahepatic cholestasis induced by 17α-ethinylestradiol via nanoformulation of Silybum marianum L. BMC Complementary Medicine and Therapies, 24(1), 51.

[14] Sherikar, A., Siddique, M. U. M., More, M., Goyal, S. N., Milivojevic, M., Alkahtani, S., ... & Nayak, A. K. (2021). Research Article Preparation and Evaluation of Silymarin-Loaded Solid Eutectic for Enhanced Anti-Inflammatory, Hepatoprotective Effect: In Vitro–In Vivo Prospect.

[15] Tung, N. T., Tran, C. S., Nguyen, H. A., Nguyen, T. D., Chi, S. C., Pham, D. V., ... & Ho, X. H. (2019). Formulation and biopharmaceutical evaluation of supersaturatable self-nanoemulsifying drug delivery systems containing silymarin. International journal of pharmaceutics, 555, 63-76.