RJNMSR
Review Journal of Neurological & Medical Sciences Review

Volume 3, Issue 2, 2025

# DEVELOPMENT AND EVALUATION OF FERULIC ACID-LOADED LIPOSOMES FOR HEPATOPROTECTIVE EFFECTS

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DOI: https:/doi.org/

| Received | Accepted | Published |
|---|---|---|
| 27 November, 2024 | 11 June, 2025 | 25 June, 2025 |

## ABSTRACT

*The following study aimed to formulate Ferulic acid loaded liposomes (FA-Lip) and to investigate their hepatoprotective effect in rats. CCL4 was used to induce hepatotoxicity. FA-Lip were prepared by thin film hydration method that was followed by extrusion technique. The physicochemical properties of FA-Lip were analyzed, including entrapment efficiency, particle size, surface charge, crystallinity, and thermal behaviour. The liposomes thus produced, exhibited a particles size of 103.1 ± 0.4 nm having 75.66 ± 3.05 percent of drug loading within its outer core. Unimodal graph was obtained, PDI 0.122 ± 0.001 and particles having a zeta potential - 6.58 ± 1.17 mV were projected using zeta sizer. The FA-Lip in vitro release profile was investigated using the dialysis bag method in phosphate buffer saline (PBS) at 7.4 pH. The release study shows pattern of biphasic release with initial faster release followed by sustained release of FA from FA-Lip. Furthermore, thermal and crystalline behavior upon estimation presented amorphous nature of prepared nanoparticles and no chemical interaction was found in FTIR analysis. Whereas, in vivo pharmacodynamic models; significant improvement in the hepatoprotective activity confirmed in FA-Lip group as compared to control group and FA dispersion. Concluded, the therapeutic potential of FA was successfully enhanced by loading in liposomes.*

*Keywords: ferulic acid, liposomes, Carbon tetrachloride, hepatotoxicity*

## INTRODUCTION

Acute liver failure (ALF) is a serious, fast developing condition marked by the start of acute liver injury and a high mortality rate. The most effective treatment for ALF is liver transplantation; nevertheless, a scarcity of donor organisations continues to be a major impediment to its use. As a result, alternative treatments are needed for this (McGill and Jaeschke, 2019).Acute liver injury (ALI) plays a key role in the progression of liver failure, which leads to severe liver dysfunction and hepatic encephalopathy. It has a significant mortality rate, which has caused clinicians to be concerned. Toxic liver injury triggers death signaling pathways in the liver, resulting in apoptosis, necrosis, in hepatocyte (Wang et al., 2019). Previous research has improved our understanding of the physiological and pathological difficulties associated with liver failure, but it has failed to explain the pathomolecular aspects that influence cell death pathways. (Trautwein and Koch, 2013).

A lot of hepatoprotective medicines are commercially available but they have serious adverse effects due to their metabolites which are non-biocompatible. As compared to commercially available drugs natural compounds are safer and have relatively better absorption, metabolism and excretion with associated benefit of metabolites that are biocompatible. However, the issue with natural drugs is that biotransformation cycles that results in their rapid metabolism so comparatively higher doses are required to for efficacious effect which limits their use. Therefore, type of delivery system is needed which can regulate the metabolism of drugs and help the delivery of the natural drug at low dose.

CCl4 is a frequently utilized chemical solvent on an industrial scale. It's a well-known hepatotoxin and the most thoroughly researched xenobiotic-induced free radical-mediated hepatotoxicity animal model. CCl4 damages the liver in a variety of ways. Enhanced lipid peroxidation as a result of increased free radical generation from CCl4 is one of the processes that contribute to hepatotoxicity. The immune system is also activated by the influx of inflammatory cells to the site of injury caused by CCl4. As a result, immune cells may be responsible for the release of pro-inflammatory cytokines such as TNF- and IL-6, which worsen hepatotoxicity through an inflammatory cycle. (Boll et al., 2001).ALI is caused in this study by an intraperitoneal injection of CCl4. The preferred model is CCl4-induced liver injury because it causes hepatic alterations similar to cirrhosis. Animal models that are suitable for examining the mechanism and pathophysiology of ALI, as well as researching alternate hepatoprotective therapies, are required.

Ferulic acid ([3-[4-hydroxy-3-methoxy-phenyl] prop-2-enoic acid) belongs to the phenolic acid class especially present in plant cell wall.t has vast range of biological and pharmacological effects. Traditionally, it is used as medicinal herb for various diseases, particularly as an anti-inflammatory and antioxidant activity. Ferulic acid possess less toxicity (Ferreira et al., 2010) and has various pharmacological functions.it has anti-inflammatory (Alam, 2019), antioxidant, antimicrobial activity (Pernin et al., 2019), anticancer (Gao et al., 2018), and anti-diabetic effect(Ohnishi et al., 2004). Ferulic acid has a free radical scavenging property, and inhibits enzymes that catalyze free radical generation and enhances scavenger enzyme activity. Figure 1 shows the structure of ferulic acid.

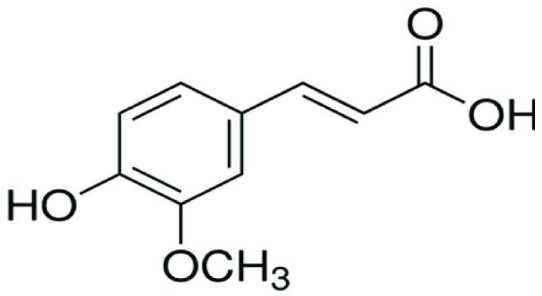


**Figure 1** Chemical structure of Ferulic Acid

Clinically available synthetic medications for the treatment of liver ailments, are not affordable, especially for patients in developing countries. These medications have the potential to induce side effects and further damage. FA can be utilized due to its safety and efficacy in various human. Despite of its efficacy and safety, FA has relative low bioavailability which is considered a major problem. Bioavailability is the major issue in the actual applicability of FA in the treatment of hepatic toxicity when taken orally dues to extensive first pass effect which results in less absorption. Also, due to fast metabolism which results to the short half-life of drug, and limited tissue distribution (Li et al., 2011). Many nano-carriers have been used to improve FA bioavailability. The current study is aimed to investigate FA loaded in liposomes could protect rats against liver injury caused by CCl4. FA is a natural medicine with a lesser solubility in water. FA's hepatoprotective potential can be boosted by encapsulating it in biocompatible and biodegradable liposome carriers. Pharmacological properties of liposomal drug delivery systems are superior to those of conventional dosage forms.

## MATERIAL AND METHODS

### *Material*

FA was generously gifted by AIMS Pharmaceuticals, Islamabad, Pakistan. Soy Phosphotidyclcholine (SPC), Cholesterol (CH), Methanol, Ethanol, Chloroform, Carbon tetrachloride, Tween 80 (TW80), Formalin was procured from Sigma Aldrich (St. Louis, MO, USA). All solvents, chemicals and reagents used were of analytical grade.

### *Preparation of FA-Lip*

The thin film hydration method was used to produce the optimal formulation of FA-CL, which was then followed by extrusion technique. (Zeb et al., 2019). In a 2:1 mixture of chloroform and methanol, FA, SPC, and cholesterol were dissolved. To obtain a thin lipid film on the round bottom flask, the organic solvent was evaporated at 40°C under decreased pressure in a rotary evaporator (Type A-1000S, Tokyo Rikakikai CO. LTD, Japan). After that, the dried lipid film was hydrated for one hour. The hydration medium was made up of phosphate buffer saline that had been heated to 65°C and had a pH of 7.4. In a bath sonicator, the vesicular solution was sonicated

for 20 minutes before being extruded 20 times over a 100 nm size polycarbonate membrane with a MiniExtruder. (Avanti Polar Lipids, Inc., Alabaster, AL, USA) The vesicles were purified using an ultrafiltration centrifugation technique to remove free FA from the FA-Lip as shown in Figure 2. The FA-Lip ( figure 3) was then lyophilized at a pressure of approximately 6 mTorr for 24 hours at a temperature below -50°C using a TFD5530 Bench-Top freeze dryer (IlShinBioBase, Republic of Korea). Before usage, the lyophilized formulation was kept at 4°C and reconstituted with deionized water.

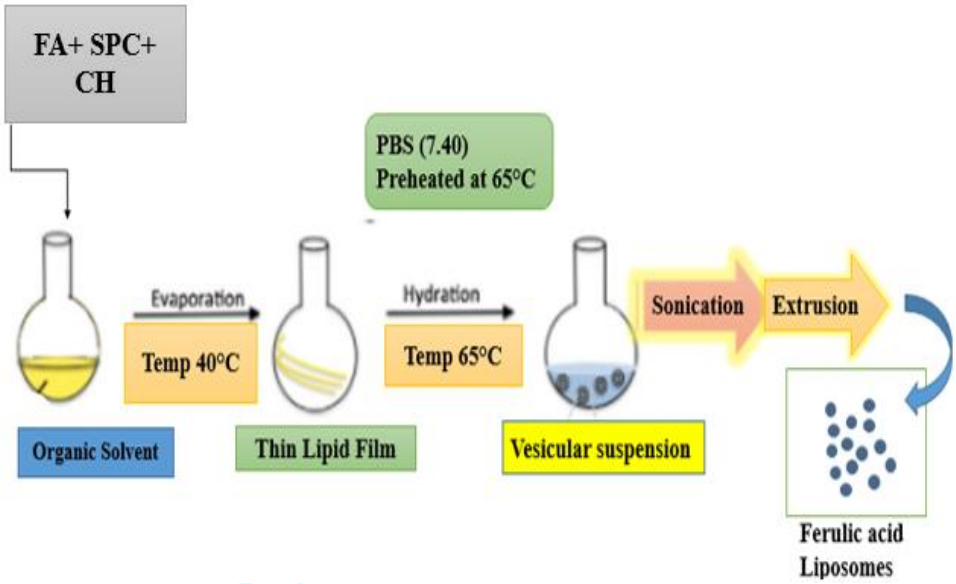


**Figure 2** Schematic representation of FA-Lip preparation method. (FA; Ferulic Acid, SPC; Soy phosphotidylcholine, CH; Cholesterol)

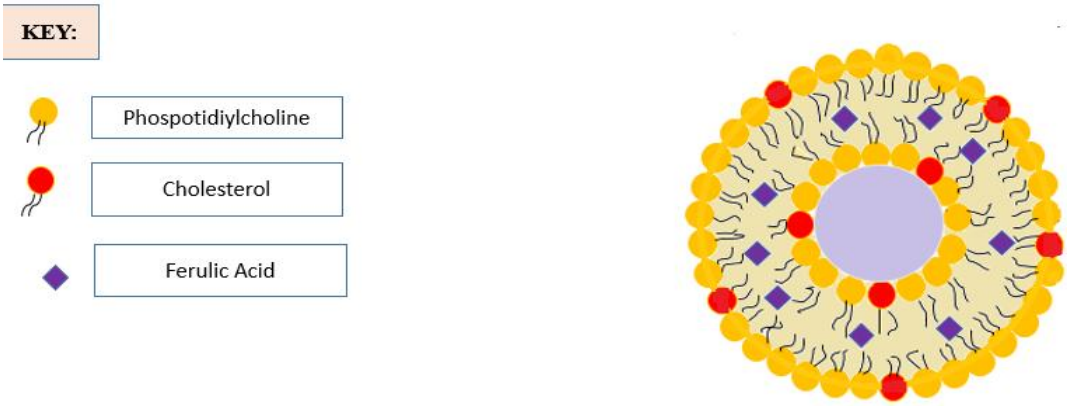


**Figure 3** Structure of FA-Lip

***Physicochemical Characterization of FA-Lip***

The particle size, zeta potential (ZP), and polydispersity index (PDI) of FA-Lip were measured using the Zetasizer ZS 90 (Malvern Instruments, Malvern, Worcestershire, UK). Electrophoretic light scattering and photon correlation spectroscopy are used in this apparatus. The zeta potential is used to determine the stability of the produced formulation. The graph depicts size distributions and PDI, with PDI ranging from 0 to 1 (Zeb et al., 2017) UV spectrophotometric analysis was used to measure the effectiveness of FA incorporation in FA-Lip. Ultrafiltration membrane pore size 0.1um was used to remove unentrapped medication. The FA content was measured using a UV system (V-530; JASCO Corporation, Tokyo, Japan) at a wavelength of 319 nm after filtered liposomes were dissolved in ethanol to the desired concentration and suitably diluted (Zeb et al., 2019). Following equation was used for the determination of entrapment efficiency:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Weight of FA entrapped In FA - Lip}}{\text{Total weight of FA added}} \times 100$$

***Thermal behavior***

By Utilizing a differential scanning calorimeter (DSC Q20; TA Instrument, New Castle, DE, USA), the thermal behavior of lyophilized FA-Lip and its solid components (FA, PC, CH) was determined. The reason why cryoprotectant was not used is to rule out the possibility of interference of thermal transition peaks. The analysis was carried out by increasing the temperature 10°C/minute achieving a maximum temperature of 200°C. Heat flow was plotted against temperature using SigmaPlot version 10.0. (Systat Software, Inc, USA) to obtain DSC curves.

***Release study of FA-Lip***

The release of FA from FA-Lip in phosphate buffered saline (PBS) at a pH of 7.4 was estimated using the dialysis bag technique (Qin et al., 2008). Dialysis membranes with a molecular weight cut-off of 3500 Da (Spectrum Laboratories, Inc., Rancho) were soaked in warm media 5 min prior to use. After those membranes were filled with FA-Lip equivalent to 5 mg of FA and sealed on both ends. Then the membranes were immersed in the release media (PBS), with the temperature and stirring remained at 37 0.5°C and 100rpm, respectively. 5ml samples were taken at predefined intervals (0.5, 1, 2, 4, 8, 12, 16, 20, 24h ) up to 24 hours, and the percent drug released was calculated using a UV-visible spectrophotometer set to 319nm to check the absorbance. Each vessel was replenished by equal amount of fresh media after every withdrawal (Zhang et al., 2010).The cumulative FA released (%) was determined using average of 3 readings (n=3). The cumulative FA released (%) data was then plotted against time (h).

***Experimental animals***

Male Sprague Dawley (S.D) rats weighing 250-350g were made available through the animal house of Riphah Institute of Pharmaceutical Sciences (Islamabad, Pakistan) in order to undertake in vivo activity in an experimental model. Before the studies, the animals were acclimatized to the

laboratory setting for a week Rats had unrestricted access to food and water, and temp maintained at 25 ±0.5°C and the relative humidity 40-60% with alternate 12h dark light cycle. In correspondence with animal welfare act, Institutional Research and Ethics Committee of Riphah Institute of Pharmaceutical Sciences approved the experiment to be conducted.

***In vivo pharmacodynamic activity***

Experimental design

Rats were divided into 5 groups (n=5). Treatments were administered intra-peritoneal. Numerous treatment groups include; Group I: normal saline; GroupII: (control group) CCL4; Group III (CCl4 +FA dispersion); Group IV (CCl4 + FA-Lip); Group V (CCl4 + Silymarin) shown in Figure 4. Hepatic injury was induced by single i.p. injection of CCL4 (0.5ml/kg) on day one in each group except for normal. The antioxidant silymarin (45 mg/kg, p.o.) was utilized as a reference standard (Eminzade et al., 2008). FA was administered equivalent to 50 mg/kg in group III and IV for 10 days i.p. According to the protocol, all of the rats were decapitated under light ether anesthesia at the end of the study. Blood samples were drawn into a heparinized tube, then separated by centrifugation. Liver samples obtained for histopathological examination were stored at 4°C in 4% formaldehyde solution to preserve their morphology.

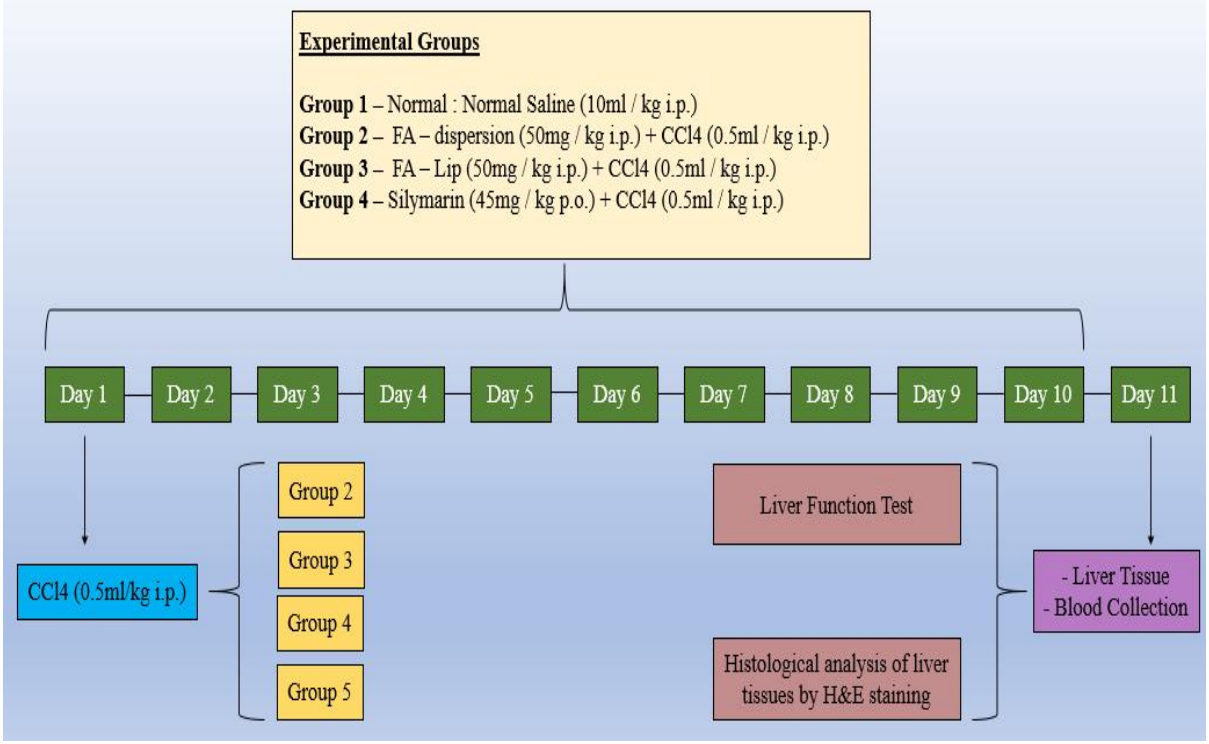


**Figure 4** Schematic representation of experimental design

Serum sample biochemical analysis

Serum samples from various groups were tested for Aspartate Transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphate (ALP) using packaged kits (Mir et al., 2010).

***Hematoxylin & Eosin (H&E) staining***

Liver sections taken immediately after dissection from the liver, fixed in 10% buffered formalin (Lin et al., 1997), dehydrated in gradual ethanol (50 to 100%), cleared in xylene, and embedded in parafine. Sections (4 to 5 um thick) were prepared and then stained with Haematoxylin and Eosin (H and E) dye for photomircoscopic observations like cell necrosis, fatty change, hyaline degeneration, ballooning and degeneration under power 40X.

***Statistical analysis***

All of the studies were done in triplicate (n=3). The standard deviations were used to present the data (SDs). To examine the difference between the control and dispersion groups, the data was statistically analysed using the student's t-test. The significance level was set below 0.05.

## RESULTS

***Optimization, composition and physicochemical properties***

Particle size, polydispersity index and zeta potential

Prepared formulations were then characterized for ZP, size and PDI. Particle size, PDI and ZP of liposomes prepared are summarized in Table 1. The optimized formulation selected based on best physicochemical parameters and entrapment efficiency of 75.66 ± 3.05% showed a particle size of 103.1± 0.4 nm, PDI was found to be 0.122 ± 0.001 and ZP of -6.58 ± 1.17 mV as shown in Figure 5A and 5B.

Table 1 Physicochemical parameters of FA-Lip

| Composition ratio | | | | Physicochemical properties | | | |
|---|---|---|---|---|---|---|---|
| Code | PC | CH | FA | PS (mm) | PDI | ZP (mV) | EE (%) |
| S1 | 9 | 1 | 3 | 125.0 ± 0.1 | 0.128 ± 0.022 | 0.392 ± 0.25 | 73.33 ± 3.05 |
| **S2** | **8.5** | **1.5** | **3** | **103.1 ± 0.4** | **0.122 ± 0.001** | **-6.58 ± 1.17** | **75.66 ± 3.05** |
| S3 | 8 | 2 | 3 | 107.8 ± 0.4 | 0.080 ± 0.004 | 7.82 ± 0.24 | 63.00 ± 2.64 |

| | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| Z-Average (d.nm): 102.8 | Peak 1: | 114.5 | 100.0 | 40.20 |
| PdI: 0.121 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.936 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality : Good | | | | |

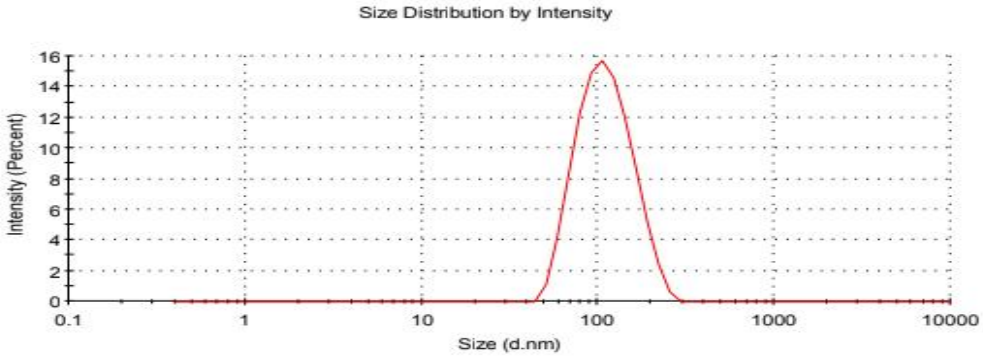


**Figure 5A** Particle size distribution curve of optimized FA-Lip

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -7.37 | Peak 1: | -7.37 | 100.0 | 7.33 |
| Zeta Deviation (mV): 7.33 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 0.0386 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality : Good | | | | |

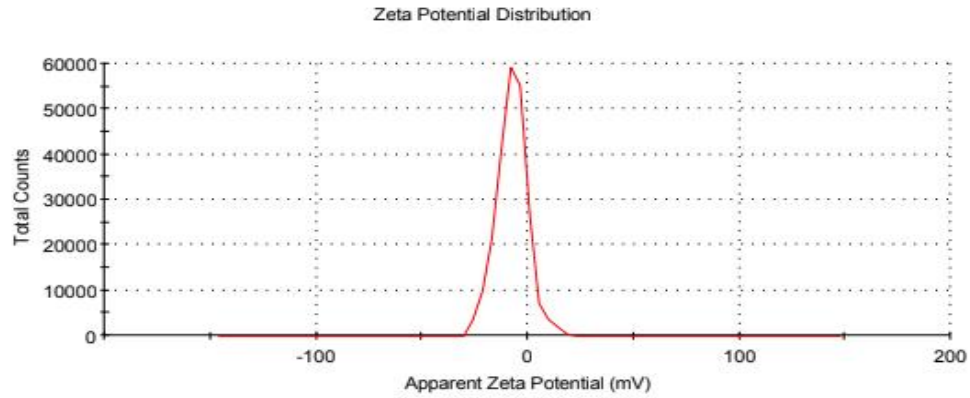


**Figure 5B** Zeta potential distribution of optimized FA-Lip.

***Thermal behavior of FA-Lip***

DSC was used to investigate the thermal phase transition of FA-Lip, CH, PC, and FA. The investigation was carried out because the polymeric form of lipid used in the preparation has an impact on the drug's release incorporation efficiency. Figure 6 depicts the transition in a loaded formulation and its solid components (FA, CH, and SPC). The results show that the sharp peak of FA has vanished in the FA-Lip, indicating that FA's initial crystalline structure has changed to an amorphous state. The entrapment capability of a formulation is determined by its thermal behavior.

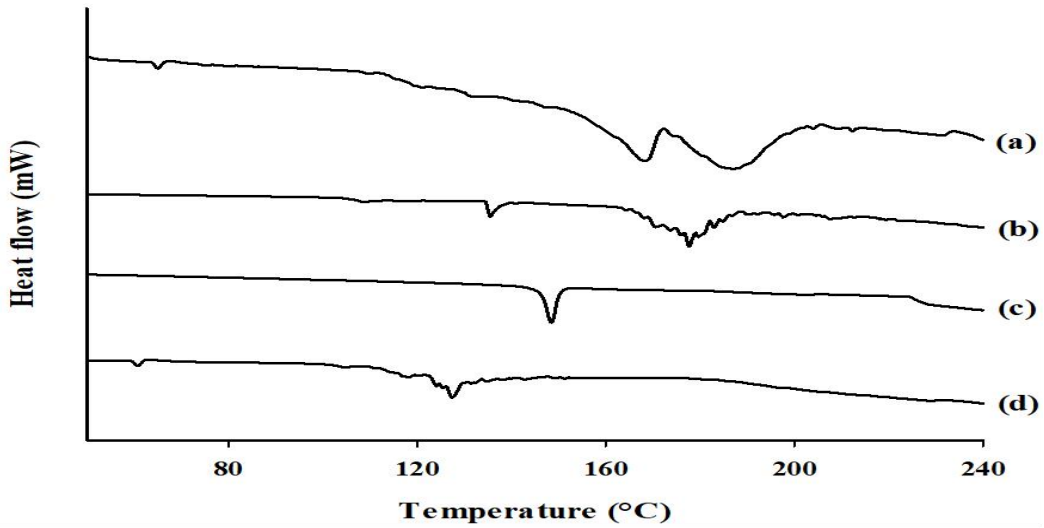


**Figure 6** DSC thermograms of FA (a), SPC (b), CH (c), FA-Lip (d).

***Release profile of FA-Lip***

FA released from FA-Lip and FA dispersion, as measured by in vitro dissolution in PBS (pH 7.4) over a 24-hour period, showed a biphasic pattern, with an initial burst release of 24.23 ± 0.75% and 63.05 ± 6.79 % from FA-Lip and FA dispersion, respectively, followed by a sustained release of 72.64± 1.85% from FA-Lip. After 24 hours, the FA dispersion was completely gone. The Figure 7 depicts a sustained release pattern.

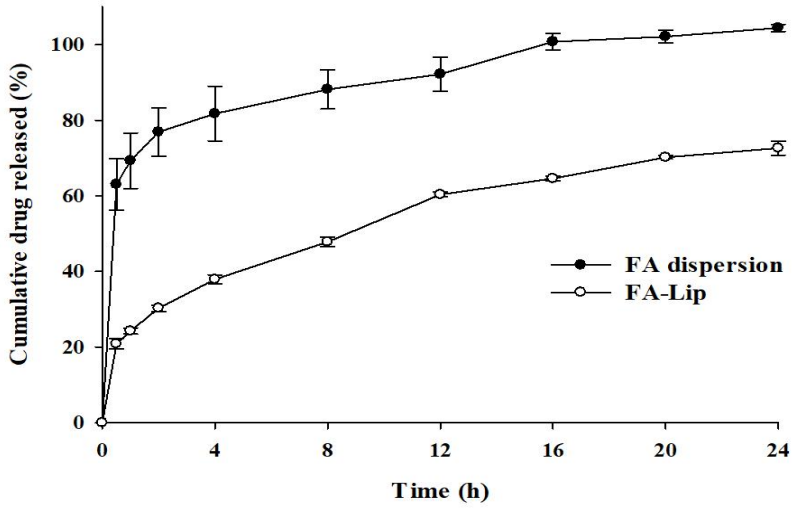


**Figure 7** In vitro release of FA from FA-Lip in phosphate buffer saline (PBS) (pH. 7.4) for 24 h at 37°C. Data is expressed as mean S.D. (n = 3).

***Biochemical investigation***

The activities of alkaline phosphate (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) in the blood, and microscopic appearance of the liver cells were all investigated. The next paragraphs present the findings.

Aspartate transaminase (AST)

Figure 8A represents the mean activity of AST in normal, control, SIL, FA-Lip and FA dispersion group. The AST in normal group was observed 15 ± 4.74 U/L , control 138.8 ± 28.04 U/L, SIL 20.8 ± 9.09 U/L, FA-Lip 22.8 ± 6.61 U/L and FA dispersion 38.6 ± 7.95 U/L respectively. It is clear from the figure that the activity of FA-Lip group was evident when compared to control group ($P < 0.005$) and greater than FA-dispersion group ($P < 0.005$) which is suggests effectiveness of the FA-Lip. The activity of FA-Lip was comparable with SIL group but enhanced effect was observed as compared to dispersion at the same dose.

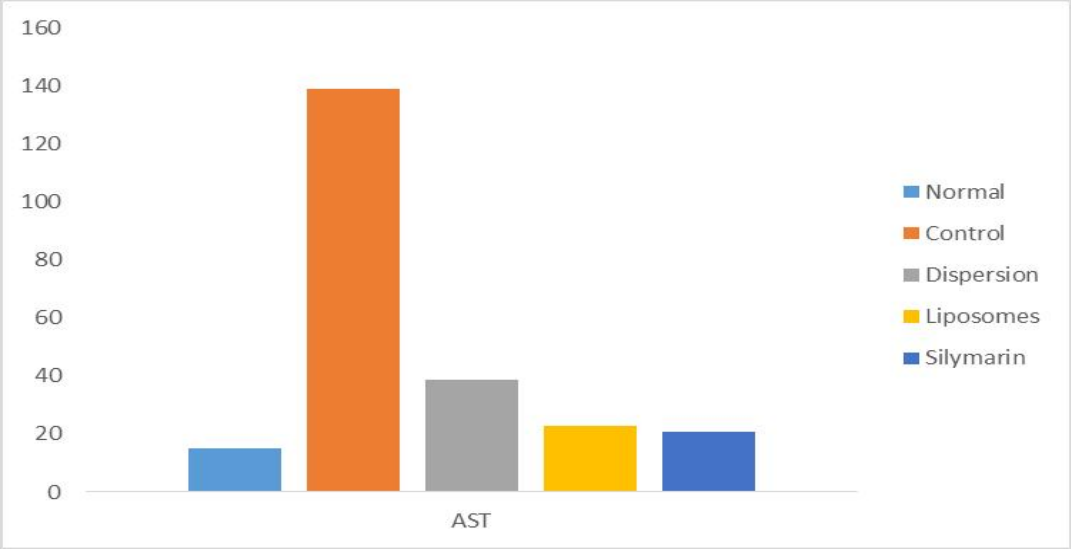


**Figure 8A** Activity of AST (U/L) in serum of various groups of rats. Data is expressed as mean S.D. (n = 5).

Alanine transaminase (ALT)

Figure 8B represents the mean activity of ALT in normal, control, SIL, FA-Lip and FA dispersion group. The ALT in normal group was observed 39.4 ± 6.22 U/L, control 146.2 ± 49.89, SIL 52.8 ± 6.99 U/L, FA-Lip 53 ± 9.51 U/L and FA dispersion 81.8 ± 13.17 U/L respectively. It is clear from the figure that the activity of FA-Lip group was evident when compared to control group ($P < 0.01$) and greater than FA-dispersion group ($P < 0.01$) which suggests effectiveness of the FA-Lip. The activity of FA-Lip was comparable with SIL group but enhanced effect was observed as compared to dispersion at the same dose.

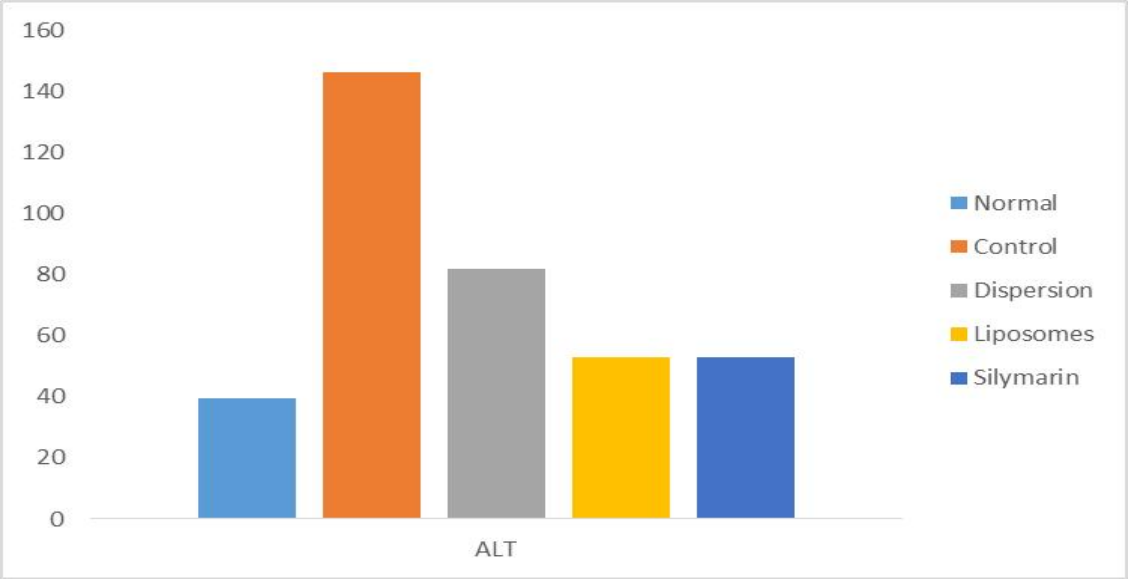


**Figure 8B** Activity of ALT (U/L) in serum of various groups of rats. Data is expressed as mean S.D. (n = 5).

Alkaine phosphatase (ALP)

Figure 8C presents the mean activity of ALP in normal, control, SIL, FA-Lip and FA dispersion group. The ALP in normal group was observed 93.6 ± 12.36 U/L control 324.8 ± 53.62 U/L, SIL 134.6± 37.89 U/L, FA-Lip 137.2 ± 4.81 U/L and FA dispersion 209.2 ± 75.69 U/L respectively. It is clear from the figure that the activity of FA-Lip group was evident when compared to control group ($P < 0.05$) and greater than FA-dispersion group ($P < 0.05$) which is suggests the effectiveness of the FA-Lip. The activity of FA-Lip was comparable with SIL group but enhanced effect was observed as compared to dispersion at the same dose.

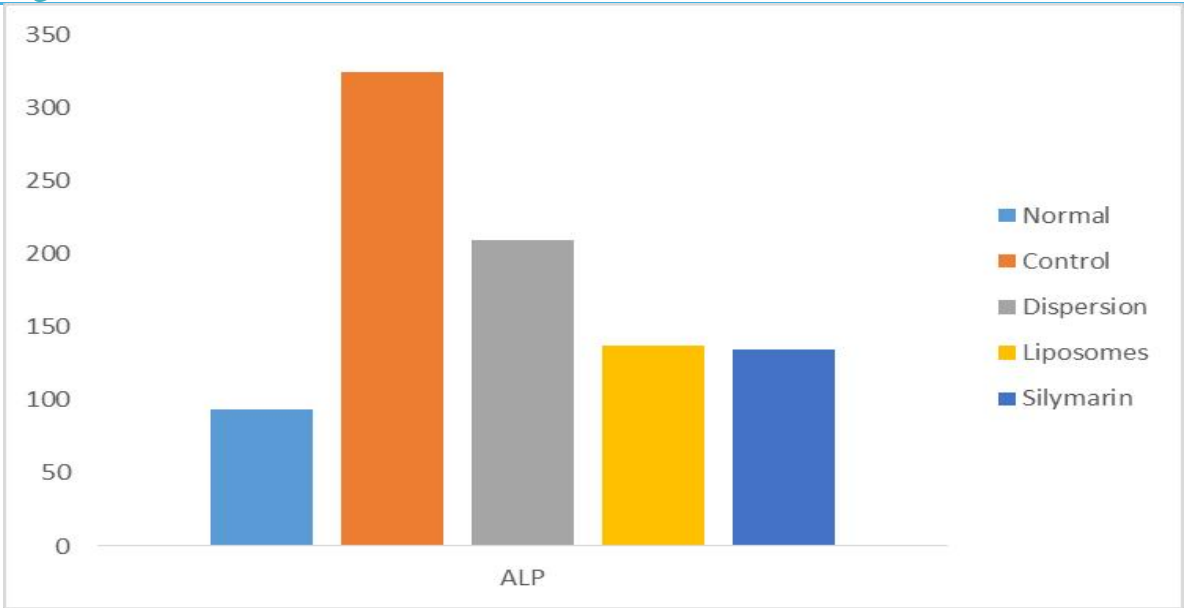


**Figure 8C** Activity of ALP (U/L) in serum of various groups of rats. Data is expressed as mean S.D. (n = 5).

Histopathological changes in liver

The control group had normal physiological structures in their liver tissue. Histopathological alterations in liver were generated by CCl4 injection, including significant ballooning of hepatic cells and infiltration of inflammatory cells. FA-Lip corrected liver injury that was induced by CCL4. The absence of FA-Lip hepatoprotection was particularly noticeable in the liver slice of rats of disease group, cellular necrosis and altered structure of hepatic cells were found. The FA-Lip had a positive effect almost identical to that of the SIL-treated group Figure 9.

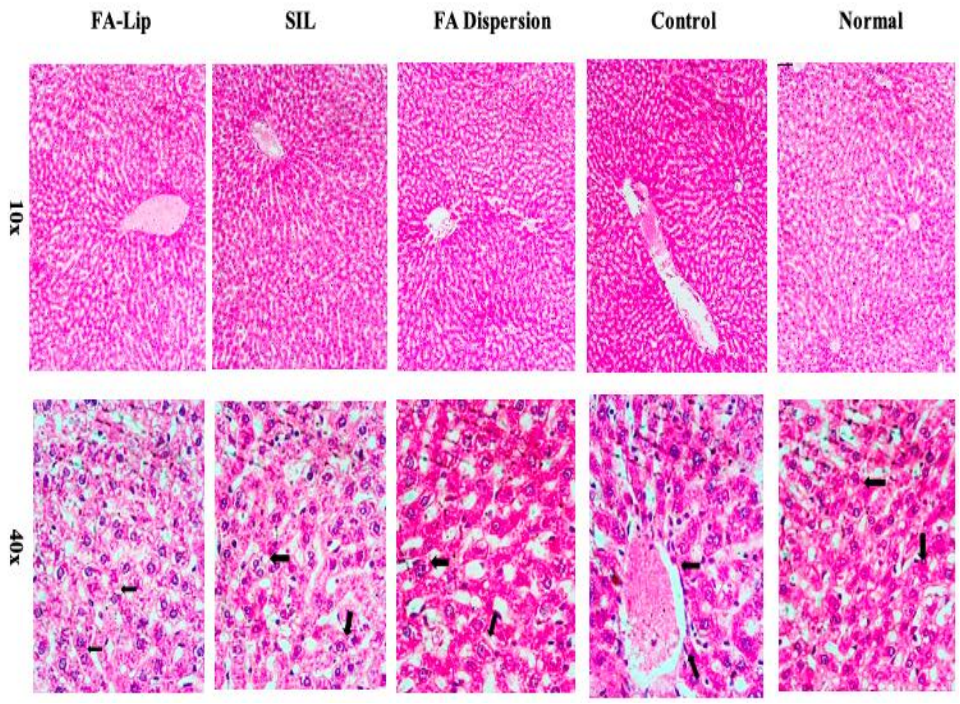


**Figure 9.** Histological micrographs (40x magnification) of liver tissues after H & E staining. CCl4-induced changes in liver histopathology of different experimental groups. Arrow heads indicate hepatocyte ballooning, necrosis and inflammation of central vein, and pericellular fibrosis in the liver parenchyma in the control group which is shown improved in treatment groups respectively.

## DISCUSSION

The hepatoprotective impact of FA and its inclusion in liposomes for the treatment of acute liver damage are investigated in this study. Because of their high EE and targeted distribution, liposomes were chosen. To evaluate the physicochemical properties of the prepared formulation, particle size, PDI, and zeta potential were evaluated. Liposomes may circulate in the blood for a long time without being entangled in the reticuloendothelial system, allowing for easier access to the liver. As a result, properly and rapidly quantifying the size of liposomes is crucial for designing novel and effective drug delivery strategies. A unimodal graph depicts an equal size distribution across the formulation, implying that particle sizes are uniform and morphologies are similar. By imparting charge on the surface of the nanocarrier, the zeta potential improves the formulation's stability and prevents aggregation within the formulation. The optimal formulation, which was chosen based on the best physicochemical properties and a 75.66 ± 3.05 %entrapment efficiency, size of particles 103.1 ± 0.4nm, a PDI of 0.122± 0.001, and a ZP of -6.58 mV ± 1.17. Because the drug is entrapped within the bilayer, which has been protected from the external environment, the liposomes sustained nature is enhanced by its uniform distribution and spherical surface (Nakhaei et al., 2021). Lyophilization has also been shown to improve stability, even when the zeta potential is low.

Thermal behavior of FA-Lip as assessed by DSC analysis showed melting properties of formulation and its components. Decrease in melting point embodies a less oriented crystalline or completely amorphous structure as shown in FA-Lip suggesting high drug incorporation.

The DSC method is a tool for measuring temperature and energy variations in phase transitions, such as the phase transitions of phospholipid bilayers from gel to crystalline liquid which indicates the degree of crystallinity of free FA contained in liposomes (Bakonyi et al., 2017).

The prepared FA-Lip was tested for release studies in vitro utilizing a dialysis membrane technique in PBS as the release media at pH 7.4. The temperature of the vessels was maintained at 37°C during the procedure, and samples were taken at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours before being analyzed spectrophotometrically at 319 nm (Hua, 2014). FA-Lip release profiles were compared to FA dispersion profiles. The release of FA from the FA-Lip indicated a brief release within the first two hours, followed by a somewhat sustained behavior that lasted for 24 hours. The formulation's initial release was 25%, with a sustained release of up to 72 % for up to 24 hours. The drug is slowly released from the lipid core as the core erodes over time. As demonstrated by DSC, the slow release of FA from the lipid core is owing to the drug's strong association with the lipid core. FA dispersion, on the other hand, showed a rapid release, with nearly total release from the dialysis bag lasting up to 24 hours (Davidson et al., 1994). DSC melting analysis was used to examine the thermal and melting behavior of lyophilized FA-Lip, as well as their solid components (FA, SPC, CH) and physical mixture. FA's distinctive endothermic peak, which correlates to its melting point, was discovered at 186°C, confirming its crystalline nature. The intensity of this peak was lowered in the case of a physical mixture, as was the melting point. This is because SPC melted before FA during heating, thus solubilizing the drug into it. A characteristic endothermic peak was also seen in response to the melting points of SPC and CH, which were found to be at 177°C° and 148 °C respectively. FA-Lip showed no melting peak, indicating that FA was either entirely entrapped in the bilayer or was concerted to an amorphous state from its initial crystalline state.

FA's effects on CCL4-induced liver injury were investigated in a hepatoprotective investigation. Our findings demonstrated that FA-Lip therapy reduced CCl4-induced toxicity and normalized serum hepatic enzymes such as AST, ALT, and ALP, which was confirmed by histological findings. In comparison to the normal group, the animals were lethargic in the disease control group and responded slowly to stimuli. This could be owing to the fact that CCl4 is an anesthetic, affecting delay in the animals' responses and other general behaviors (Al-Harbi et al., 2014). The normal group presented the serum levels AST, ALT and ALP levels of 15± 4.74 U/L, 39.4±6.22 U/L, 93.6±12.36 U/L whereas disease control group showed the same values as 138.8 ± 28.04 U/L , 146.2 ± 49 .89 U/L, 324.8 ± 53.6 4 U/L respectively. Animals in this group appeared ill looking, less hungry as compared to normal group and lost body weight. These results show that CCl4 hepatotoxicity was efficiently induced in diseases group with markedly elevated levels of hepatic markers. The FA-Lip group showed the serum AST, ALT and ALP levels of 22.8 ± 6.61 U/L 53 ± 9.51 U/L, 137.2 ± 4.81 U/L respectively which showed close correlation with SIL group which is a reference standard (Gillessen and Schmidt, 2020). The SIL group was presented with the serum levels of AST, ALT and ALP 20.8 ± 9.09 U/L, 52.8± 6.99 U/L, 134.6 ± 37.89 U/L, respectively whereas the dispersion group AST, ALT and ALP levels were 38.6 ± 7.95 U/L, 81.8 ± 13.17 U/L, 209.2 ±75.69 U/L. Comparison of FA-Lip group and SIL group presents not much significant difference. However, a marked improved activity is seen in FA-Lip group as compared to FA dispersion at the same dose which justifies the drug loading into liposomes. The animals in this

group responded actively, maintained food and water intake and no marked weight loss was seen. Briefly, treatment with, SIL and FA-Lip improved CCl4 induced changes in biochemical tests. Dispersion group also improved the changes induced by CCL4 but was comparatively less.

Upon histological examinations, normal group had normal morphological structures in their liver tissue Figure 9 Histopathological alterations in the liver were generated by CCl4 injection, including significant necrosis of central lobule, ballooning of hepatocyte, in the portal tube and sinusoid in the disease control group. There were minimal lesions in FA-Lip group and fatty change was remarkably decreased which reveals that FA was effectively delivered when incorporated in liposomes and protected liver. Even though decrease in body weight was observed but average decrease of absolute body weight was less than dispersion group. Histopathological findings mirrored biochemical improvements after FA-Lip therapy. FA-Lip treatment reversed the CCl4-induced hepatic damages. It was visible in the liver segment by the absence of cellular necrosis and cellular structure restoration.

## CONCLUSION

Liposomes were used to transport a natural drug and ensure that it was delivered effectively. FA-Lip were formulated by thin film hydration and were further extruded. Lipids were biocompatible and biodegradable, and the results were reproducible. On zeta analysis, FA-Lip showed a homogeneous particle size distribution with a particle size of 103.1 ± 0.4 nm with a spherical morphology. The absence of any chemical interactions within the formulation was confirmed by FTIR analysis, while DSC revealed a normal amorphous liposome with adequate drug incorporation. In vitro release tests revealed sustained release of FA. Biochemical testing and histological examinations revealed that in vivo pharmacodynamics data showed improved hepatoprotective effects. Concluding FA-Lip can effectively be used as not only for incorporating and sustained release of FA but they are also efficacious for hepatoprotective effects as demonstrated by *in vivo* analysis.

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