Computational Assessment of Nanoencapsulated Liposome-Insulin for Diabetes-Associated Liver Protection

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Abstract: This study evaluates the therapeutic potential of nanoencapsulated liposome–insulin as an advanced drug delivery system for mitigating diabetes-associated liver dysfunction. The formulation was prepared using the solvent injection method and characterized by UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and field emission scanning electron microscopy (FESEM), confirming successful encapsulation, structural integrity, and nanoscale particle size ranging from 45 to 177 nm. An in vivo model was established using alloxan-induced diabetic male rats divided into control, untreated diabetic, and treated groups. Liver function was assessed through serum biomarkers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), alongside histopathological examination. Diabetic rats exhibited significantly elevated enzyme levels, indicating hepatic damage. Treatment with liposome–insulin resulted in a marked reduction in AST and ALT levels, while ALP remained elevated. Histological findings confirmed improved hepatic architecture, reduced necrosis, and decreased inflammatory infiltration in treated animals. The observed effects are attributed to enhanced insulin stability, improved bioavailability, and more efficient cellular delivery provided by the liposomal system. These findings suggest that nanoencapsulated liposome–insulin represents a promising strategy for improving therapeutic outcomes and reducing liver complications associated with diabetes.

1 INTRODUCTION

Diabetes is a metabolic disorder-affecting human, where the body either does not produce insulin or does not use it in a proper way [1]. There are two main types of diabetes: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). When comparing type 1 diabetes with type 2, noticeable differences are observed. Type 1 diabetes is an autoimmune disorder that destroys pancreatic beta cells and prevents insulin secretion, while in type 2 diabetes, insulin levels rise and insulin resistance appears in the cells. Gestational diabetes is considered a subtype of diabetes [2], [3].

Medications are principally utilized to save life and alleviate symptoms, with secondary objectives including the prevention of long-term diabetes problems and the enhancement of life expectancy by mitigating numerous risk factors. Insulin replacement therapy is the principal treatment for those with type 1 diabetes, but dietary and lifestyle adjustments are fundamental for the treatment and management of type 2 diabetes [4].

According to epidemiological studies, the number of individuals diagnosed with diabetes is projected to increase from 425 million in 2017 to 629 million by 2045 [5]. Consequently, better management of diabetes has become necessary. Nanomedicine has recently made great strides in bettering medical condition treatment [6]. The fundamental focus of nanomedicine is the use of drug-loaded nanoparticles that can regulate their release through several

processes affected by environmental factors. Research has demonstrated that controlled drug release can lead to more effective targeted therapies, more accurate drug delivery, and better therapeutic outcomes [7]-[9].

When it comes to managing diabetes, nanoparticles have multiple uses, but one of the most important is to decrease the likelihood of hypoglycemia by triggering insulin release in reaction to changes in glucose concentration, thereby reducing the risk of hypoglycemia [10], [11]. A further goal is to prevent insulin breakdown in the digestive tract, allowing for oral administration and avoiding daily injections [12]. Nanoparticles are biocompatible and biodegradable spherical entities that include either conventional or biological therapeutics, including peptides and nucleotides. They are uniquely characterized by their nanoscale size, which are highly typically ranging from 100-300 nanometers, and can be administered orally or via injection. These particles seemingly act as drug carriers, protecting the drug from eco- conditions at the site of administration, transporting their therapeutic load to targeted body sites, and releasing the drug in response to environmental stimuli at the site of delivery [13], [14].

Of such nanoparticles are liposomes, which are noticeably vesicles composed of phospholipid bilayers surrounding an aqueous phase. Liposomes are undoubtedly apt for hydrophilic drugs dissolved in water, as well as hydrophobic drugs dissolved in the lipid bilayer [15], [16]. The liposomal membrane safeguards encapsulated pharmaceuticals from oxygen, moisture, and light, allowing for regulated and localized release in precise locations [17]. Liposomes can enhance the physicochemical properties and onset of action of drugs while reducing their toxicity. Due to their biodegradability, biocompatibility, and stability in colloidal solutions, liposomes meet the criteria for effective drug carriers [18].

2 MATERIALS AND METHODS

The solvent injection method was used to prepare the liposomes, employing ethanol and chloroform as the medium. A total of 60 mg of soy lecithin and cholesterol at a mass ratio of 4:1, 40 mL of chloroform, and insulin were dissolved in the mixture, alternating with lecithin and cholesterol. The mixture was stirred at 400 rpm at a temperature of 23 °C for 20 minutes to ensure complete dissolution of the lipid mixture in the medium. An injection nozzle (2.5 mm diameter) was placed at a depth of 1.5 cm below the water surface in a beaker, with the water temperature maintained at 60 ± 2 °C using a temperature control device. To demonstrate the effect of evaporation temperature, the lipid-dissolved medium was injected into water by driving its surface in the moving reservoir toward the injection nozzle. The injection speed was 10 mL/min, controlled by a manual flow-regulating valve, while monitoring the rate of decrease of the medium level inside the moving reservoir. The injected medium evaporating in water, and the resulting liposome emulsion was perfectively filtered by making use of a vacuum filter and a 0.4 μm filter paper to strictly remove any excess lipids [19].

2.1 Characterization of the Nanoencapsulated Lliposome-Insulin

The nanoencapsulated liposome-insulin was uniquely characterized by effectively and meticulously making use of ultraviolet-visible spectroscopy (UV-Vis 1800). A double-beam ultraviolet-visible spectrophotometer (PD-303 UV) was effectively employed to meticulously detect the surface plasmon resonance (SPR) property of liposome-insulin particles at room temperature. Fourier-transform infrared spectroscopy (FTIR) was also used to efficiently study the molecular vibrations of the sample molecules within the spectral range of 1000–3500 cm^{-1}. In addition, (FESEM, Jeol JSM-6460 LV) was meticulously used to efficiently examine the morphology and structure of the nanoencapsulated particles. In addition, Figure 1 illustrates the research study plan, which went through the stage of preparing the coated nanomaterials and confirming their unique physical properties through examination, as well as dosing experimental animals and conducting physiological tests.

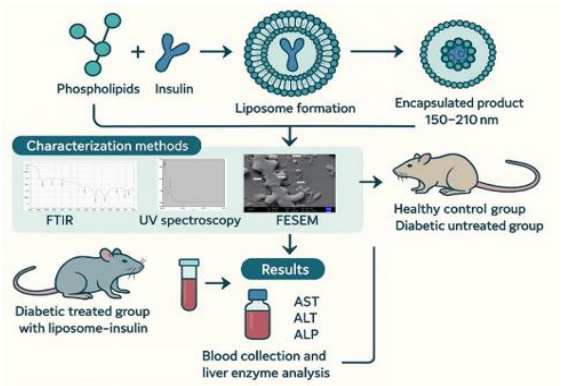

Figure 1: Model illustrating the workflow and experimental procedures.

2.2 Animals

An aged 5 - 6 month male Swiss albino rats weighing 167–218 g was caged in closed cages in the animal house of the Biotechnology Research Center. They were effectively maintained under laboratory-controlled settings at 25 °C and provided with pellet food and tap water. Diabetes was induced by alloxan at a dose of 150 mg/kg. Alloxan was used to induce diabetes in rats due to its selective toxicity to pancreatic β-cells, leading to insulin deficiency and hyperglycemia, thereby providing a reliable experimental model to evaluate antidiabetic therapies. The animals were divided into three groups, each consisting of five rats: the first group was a non-diabetic control, the second was a diabetic control, and the third was diabetic and treated with the nanoencapsulated liposome-insulin. Blood samples were meticulously collected after 4 weeks of treatment by direct cardiac puncture under the influence of ether anesthesia, and serum was and effectively used to meticulously analyze liver enzymes, including:

1) Estimating the enzymatic activities of serum aminotransferases, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). The method used was based on the estimation of the amount of pyruvate and oxaloacetate released through their reaction with 2,4-dinitrophenylhydrazine [20].
2) Estimating the serum Alkaline Phosphatase (ALP) activity. This method was based on the estimation of the amount of phenol released through its reaction with 4-aminoantipyrine [21].

2.3 Histological Study

All animals were dissected for histological examination. The liver was excised and fixed in 10% neutral formalin solution. Small samples were taken from the kidneys and processed by using conventional histological methods, including dehydration with ethyl alcohol, clearing with xylene, and embedding in hot paraffin wax. The paraffin blocks were sectioned at 5 μm thickness by using a microtome, and the slides were stained with Harris's hematoxylin and eosin (aqueous) [22]. The pathological histological samples were examined using a light microscope.

2.4 Statistical Analysis

A one-way analysis of variance (ANOVA) was utilized in order to analyse the data. Utilizing Duncan's multiple range test, the level of significance that was accepted was $P < 0.05$ [23]. This test was utilized to assess the differences that existed between the groups that were stated.

3 RESULTS AND DISCUSSION

The absorption bands of the ultraviolet (UV) spectrum for the nanocarrier insulin-liposome were identified at wavelengths between 200–800 nm, representing the absorption peaks of the nanoencapsulated insulin-liposome. Figure 2 shows three absorption peaks: the first at 272.00 nm, which indicates the presence of aromatic amino acids in insulin; the second at 228.50 nm, which reflects the peptide bonds in the insulin chain, indicating the stability of the protein's secondary structure and the absence of degradation; and the third at 216.50 nm, which is associated with the phospholipid component of the liposome. These three peaks confirm the successful encapsulation of insulin within the liposome and the formation of a stable complex in which both insulin and the liposome retain their properties. These findings are consistent with those reported by [24].

Figure 3 presents the FTIR spectrum of the nanocarrier liposome-insulin, appearing as peaks at different wavelengths. The peak at 3390.86 cm^{-1} corresponds to the N-H or O-H groups, representing vibrations of hydroxyl or amine groups, which indicates interaction between insulin and the liposome. The peak at 2927.94 cm^{-1} corresponds to aliphatic groups, showing the presence of alkyl chains in the structural composition. The peak at 2397.52 cm^{-1} arises from CO_2 interference or the reactive environment. The peak at 1639.49 cm^{-1} falls within the amide the first district (C=O stretching), which is undoubtedly a strong index of insulin retaining its secondary structure. The peak at 1359.82 cm^{-1} is corresponding to C-H bending vibrations, representing methyl groups in insulin's structure. The peak at 1130.29 cm^{-1} stands for C-O-C vibrations, functioning as reliable proof of interaction among the functional groups of insulin and the liposome. The peak at 829.39 cm^{-1} results from vibrations in the nanocarrier due to insulin–liposome interactions. Lastly, the low-frequency peaks at 621.08 and 526.57 cm^{-1} point out the steadiness of both insulin and the liposome, which undoubtedly boosts up the stability of the nanocarrier. This spectrum efficiently uncovers clear chemical bonding between liposome and insulin, and the appearance of amide peaks affirms that insulin retained its structural integrity during the

encapsulation process. The indication accompanying low frequency peaks further tremendously maintain the success of the encapsulation. These results are in line with those reported by [25].

Furthermore, Figure 4 of the liposome-insulin nanocarrier shows irregularly shaped nanoparticles, with tubular and branched quasi-spherical structures. The measured diameters of the nanoencapsulated liposome-insulin ranged from 45.42 to 177.22 nm, which falls within the appropriate nanoscale range. The surface appeared rough, and the particle distribution was heterogeneous due to surface charge and inter-particle attraction effects. These findings are consistent with the results of the study conducted by [26].

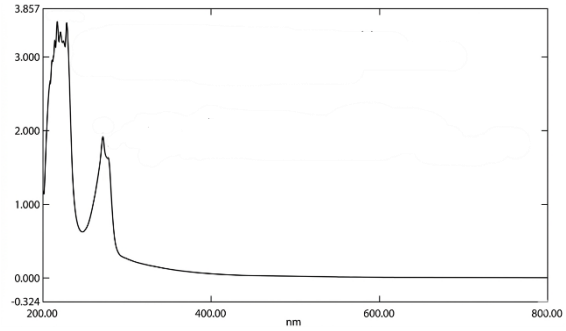

Figure 2: UV-visible spectral analysis of the liposome nanocarrier.

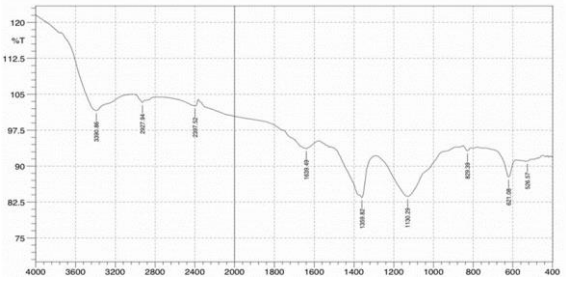

Figure 3: Infrared (FTIR) spectral analysis of the liposome–insulin nanocarrier.

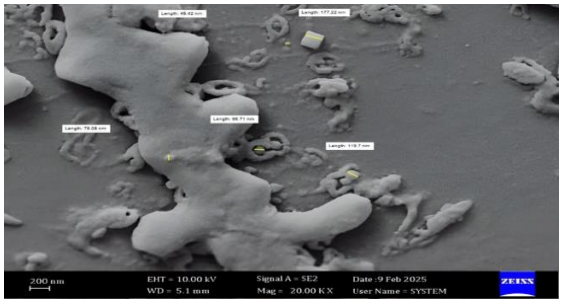

Figure 4: Scanning electron microscope image of the liposome–insulin nanocarrier.

3.1 Biochemical Tests

Table 1 shows the current results, indicating a significant increase in AST enzyme activity in the diabetic control group induced with alloxan compared to the healthy control group. The results also demonstrated a significant decrease in AST enzyme levels in the blood of animals treated with the nanocarrier liposome-insulin compared to the diabetic control group. The findings of this study agree with previous research [27]. Showing that alloxan-induced diabetes causes an increase in liver AST enzyme activity in rat serum. This increase is attributed to cellular liver damage, which leads to elevated enzyme levels [28]. The rise in AST levels is also due to hypertrophy of hepatocytes and stimulation of the endoplasmic reticulum to produce a greater amount of the enzyme to match cell size [29]. Elevated AST concentrations in diabetic patients result from liver weakness and leakage from tissues into the serum [30].

On the other hand, the current study observed a decrease in AST enzyme levels in the group treated with the nanoencapsulated liposome-insulin. This study agrees with the findings of [31], which investigated the use of liposomes in rats with streptozotocin-induced diabetes. Insulin-loaded liposomes contribute to improving liver function associated with AST enzyme levels through a primary mechanism: enhancing insulin delivery and reducing insulin resistance. They protect insulin from early degradation and facilitate more effective delivery to cells, enhancing the body's response to insulin and lowering resistance. This improves blood glucose regulation and reduces oxidative stress and diabetes-related hepatic inflammation [32], [33]. Additionally, it mitigates liver cell damage by regulating glucose levels and reducing inflammation, thereby decreasing cellular injury and limiting AST leakage from damaged cells into the bloodstream. Consequently, reduced AST levels reflect improved liver function [34].

Table 1: Liver enzyme levels in male rats with diabetes treated with liposome–insulin.

Experimenta groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	± 105.8c 6.84	69.50a 8.62 ±	343.0a 26.94 ±
Diabetic control	± 678.5a 155.1	179.3a 67.20 ±	444.0a 202.7 ±
Diabetes+Liposome	159.3bc 8.49 ±	± 73.0a 7.86	720.8a 42.43 ±

The results also showed that alloxan-induced diabetes caused an increase in ALT enzyme levels in

the serum of diabetic rats, consistent with findings reported by [35]. The elevation in ALT is primarily due to the production of free radicals, which cause hepatocyte breakdown and necrosis, releasing these enzymes into the bloodstream. Free radicals also contribute to liver tissue fibrosis and damage, leading to the loss of enzyme receptors on epithelial cells lining the bile ducts and around the central vein, thereby increasing enzyme release from cells [36]. This ALT increase in diabetic rats is attributed to beta-cell damage in the pancreas induced by alloxan [37]. The liposome envelope also enhances the stability of insulin, preventing its premature degradation and ensuring that a greater proportion of the drug reaches the bloodstream in an active form []. The liposome envelope also enhances cellular uptake, ensuring targeted distribution and direct delivery of insulin to the cells that need it, which enhances its therapeutic effect and reduces systemic exposure [39].

Furthermore, the results indicated a significant increase in ALP enzyme levels in the diabetic control group induced with alloxan compared to the healthy non-diabetic group. The results also revealed that the ALP levels were significantly elevated in diabetic rats treated with the nanoencapsulated liposome-insulin compared to healthy controls [40]. This study demonstrated that alloxan-induced diabetes caused an elevation in ALP enzyme levels in the serum of male rats. These findings are consistent with those reported by [41], which indicated that increased ALP levels serve as a marker of liver damage, primarily due to hyperglycemia and the formation of free radicals, and secondarily due to the effects of diabetes and alloxan [42]. The rise in ALP levels may also result from the breakdown of tissues, such as liver tissue, caused by metabolic dysfunction, reduced bile secretion, or increased enzyme accumulation in tissues [43]. Additionally, it has been reported that approximately 54% of diabetic patients exhibit pancreatic beta-cell involvement. Since the bile duct, through which ALP is secreted, passes through the pancreas, pancreatic injury can lead to elevated ALP levels due to disruption of bile flow [44].

3.2 Histological Study

Figure 5 shows a transverse histological section of the liver of male rats from the healthy non-diabetic control group. The liver is composed of several lobules, each containing a central vein surrounded by polygonal hepatocytes arranged in cords. Between these cords are blood-filled spaces called sinusoidal capillaries, which contain some specialized cells known as Kupffer cells.

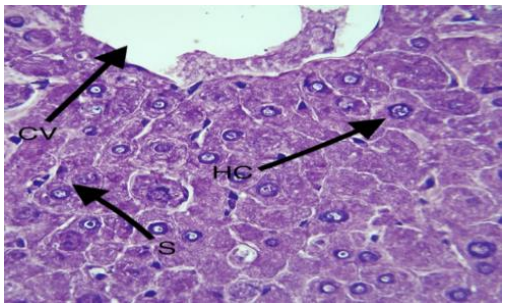

Figure 5: Transverse section of a healthy non-diabetic rat liver showing a normal central vein (CV), hepatocytes (HC), and sinusoids (S). (H&E, 40X).

Figure 6 shows a transverse histological section of the liver of a male rat from the diabetic control group induced with alloxan. Multiple areas of the liver lobules show congestion in the central veins and venous sinusoids, irregular sinusoidal structure due to focal destruction, necrosis in some hepatocytes, pyknosis of nuclei, membrane degeneration, presence of mononuclear inflammatory cells, and cytoplasmic vacuolar degeneration, compared to the healthy control group.

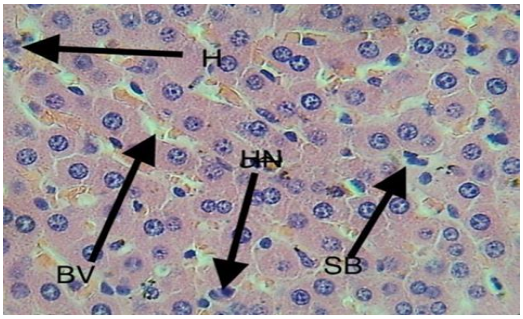

Figure 6: Transverse section of the liver of a diabetic untreated rat showing hepatocyte necrosis, nuclear pyknosis (HN), vascular congestion (BV), and sinusoidal dilation (SB). (H&E, 20X).

Figure 7 shows a transverse histological section of the liver from a diabetic male rat. A significant improvement in liver tissue was observed after treatment with the nanoencapsulated Liposome–Insulin at the cellular level. The results appeared to be nearly normal, with the hepatocytes restored to their cord-like arrangement. Nuclear division and hepatocyte proliferation were also observed, resembling the normal parenchymal structure of the liver.

The results of the current study indicate that alloxan-induced diabetes causes changes in the liver of male rats, consistent with the findings of [45]. In rats, elevated blood glucose levels following alloxan injection lead to mild sinusoidal dilation, which may

be caused by impaired venous flow at the level of the hepatic vein or the inferior vena cava. Sinusoidal dilation may also result from increased pressure in the hepatic portal vein [46].

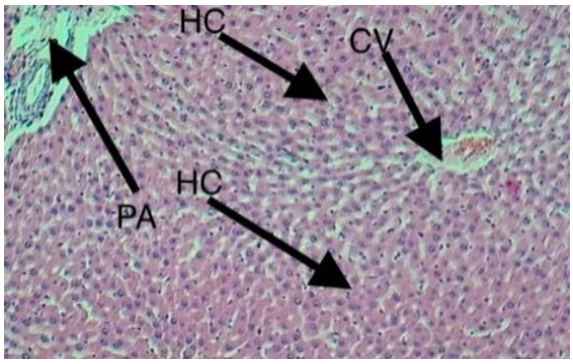

Figure 7: Transverse section of the liver of a diabetic rat treated with the nanoencapsulated liposome–insulin showing the central vein (CV), portal area (PA), portal vein, hepatic artery, bile duct, and hepatocytes (HC). (H&E, 100X).

Hepatocyte necrosis was also observed, agreeing with the findings of [47], [48]. Showing that diabetic rats can experience hepatocyte necrosis due to impaired hepatic blood supply caused by arterial thrombosis and occlusion of the hepatic artery, leading to hypoxia. This oxygen deficiency triggers the release of lysosomal enzymes and other secretory products into the blood, explaining the hepatocyte necrosis and damage [49], [50].

The presence of multiple inflammatory areas and cytoplasmic vacuolation results from liver cell damage due to immune mechanisms or the toxic effects of alloxan, including oxidative stress caused by free radical accumulation, which damages hepatocytes and oxidizes cell and mitochondrial membranes, eliciting inflammatory and immune responses [51], [52].

Blood congestion was observed in some regions, attributed to impaired blood drainage from venous obstruction, leading to impaired hepatic parenchymal blood flow, as noted by [50]. The occurrence of blood congestion because of diabetes in this study is consistent with the findings of [53], [54].

4 CONCLUSIONS

This study provides comprehensive evidence that nanoencapsulated liposome–insulin constitutes a promising and effective drug delivery system for improving liver function under diabetic conditions. The formulated nanocarrier exhibited appropriate physicochemical characteristics, including nanoscale size distribution, structural stability, and successful insulin encapsulation, as confirmed by spectroscopic and morphological analyses.

In vivo results demonstrated that administration of liposome–insulin significantly reduced serum AST and ALT levels compared to untreated diabetic controls, indicating attenuation of hepatocellular injury. Histopathological examination corroborated these findings, revealing substantial restoration of liver architecture, reduced necrosis, and improved cellular organization. However, the persistence of elevated ALP levels suggests that the therapeutic effect may be partial and pathway-specific, requiring further investigation.

The improved therapeutic performance of the nanoformulation can be attributed to enhanced insulin protection from degradation, increased bioavailability, and more efficient delivery to target tissues, leading to better metabolic regulation and reduced oxidative and inflammatory damage in hepatic cells.

Overall, the findings support the potential application of liposome-based insulin delivery systems as an advanced therapeutic strategy for managing diabetes-related liver complications. Future studies should focus on mechanistic investigations at the molecular and genomic levels, long-term efficacy and safety assessments, and comparative analyses with other nanocarrier systems to further validate and optimize this approach for clinical translation..

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