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Original article

Development and optimization of solid lipid nanoparticles loaded with Glimepiride for enhancement of pharmacokinetic and therapeutic applicability

Short title: In-silico pharmacokinetic and therapeutic applicability of developed Glimepiride SLNs

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Thanks & Regards

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Abstract

Diabetes mellitus, particularly type 2 diabetes, is a growing global health concern. Glimepiride, a widely used antidiabetic drug, faces challenges related to its poor aqueous solubility and short half-life, impacting its pharmacokinetics and therapeutic efficacy. In this study, solid lipid nanoparticles (SLNs) of Glimepiride were developed and evaluated through different analytical techniques. α-amylase and α-glucosidase inhibitory assay were performed to determine the anti-diabetic effect of Glimepiride while in-silico ADME analysis was performed to determine the pharmacokinetic behavior of Glimepiride. Thereafter, network pharmacology analysis of Glimepiride was performed to determine multi-mechanistic and therapeutic response in context to the diabetes and other pathophysiological response. In this context, have emerged as a promising drug delivery system for improving the bioavailability and sustained release of poorly water-soluble drugs. This study focuses on the development and optimization of Glimepiride-loaded SLNs to enhance its pharmacokinetic profile and therapeutic applicability. A comprehensive exploration was undertaken to encapsulate Glimepiride within SLNs using various lipid matrices and surfactants. The resulting SLNs were characterized for their particle size, polydispersity, zeta potential, and drug

encapsulation efficiency. The formulation was further assessed for in vitro release kinetics and stability. The pharmacokinetic and pharmacodynamic profiles of Glimepiride-loaded SLNs were evaluated in animal models, shedding light on their potential to enhance drug bioavailability and maintain prolonged antidiabetic effects. This research offers insights into the development of advanced drug delivery systems to address the limitations of conventional antidiabetic drugs. The results of this study indicate that Glimepiride-loaded SLNs have the potential to revolutionize diabetes management by providing improved therapeutic outcomes and patient compliance.

Keywords: Glimepiride, Solid Lipid Nanoparticle, In-silico docking analysis, Network pharmacology.

1. Introduction

The field of pharmaceutical research has witnessed remarkable advancements in drug delivery systems aimed at optimizing the pharmacokinetics and therapeutic efficacy of various medications. One such innovative approach is the development and optimization of solid lipid nanoparticles (SLNs) as carriers for drugs [1,2]. In this study, we explore the potential of SLNs as a drug delivery system for Glimepiride, a well-known oral antidiabetic agent. The objective is to enhance its pharmacokinetic profile and therapeutic applicability, addressing the challenges associated with the treatment of type 2 diabetes mellitus [1].

Diabetes, a chronic metabolic disorder characterized by hyperglycemia, poses a global health challenge. Its management often involves long-term drug therapy. Glimepiride, a third-generation sulfonylurea derivative, is a commonly used antidiabetic drug. It functions by stimulating insulin secretion from pancreatic β-cells, thus improving glucose utilization. Despite its efficacy, Glimepiride faces some limitations. The drug exhibits poor water solubility, which affects its bioavailability. Additionally, its short half-life necessitates frequent dosing, leading to patient non-compliance. These challenges underscore the need for innovative drug delivery systems to enhance the therapeutic outcomes of Glimepiride [1,3].

Solid Lipid Nanoparticles (SLNs) represent a promising approach to drug delivery. These nanoparticles are composed of biocompatible and biodegradable lipids. The solid lipid matrix provides an excellent environment for the encapsulation of a wide range of hydrophobic and hydrophilic drugs. SLNs offer several advantages over traditional drug formulations. They enhance drug solubility, improve stability, and enable controlled and sustained drug release. Moreover, SLNs can be engineered to provide targeted drug delivery to specific sites, minimizing side effects and maximizing therapeutic benefits [2,4].

Glimepiride, being a hydrophobic compound, is an ideal candidate for SLN-based drug delivery. By encapsulating Glimepiride within SLNs, we can overcome its poor water solubility and achieve controlled release kinetics. This approach not only ensures a more consistent and prolonged therapeutic effect but also simplifies the dosing regimen, leading to better patient compliance. Furthermore, the use of SLNs can potentially minimize the risk of hypoglycemia, a common concern associated with Glimepiride therapy [4–6].

The primary objective of this study is to develop Glimepiride-loaded solid lipid nanoparticles with optimized characteristics. These nanoparticles should have a well-defined particle size, a tailored surface charge, and a high drug-loading capacity. Such attributes are essential to ensure the desired drug release profiles and overall therapeutic efficacy. The study also aims to evaluate the in vitro and in vivo performance of Glimepiride-loaded SLNs, including their pharmacokinetics, tissue distribution, and antidiabetic effects [4,7,8].

The development and optimization of Glimepiride-loaded SLNs have the potential to significantly improve the treatment of type 2 diabetes. This research may lead to a more patient-friendly drug formulation that enhances drug solubility, prolongs drug release, reduces dosing frequency, and minimizes side effects. Additionally, the study's findings will contribute to the growing body of knowledge on SLN-based drug delivery systems, offering insights that can be applied to other hydrophobic drug compounds facing similar challenges [9,10].

However, this study endeavors to harness the advantages of SLNs to enhance the pharmacokinetics and therapeutic applicability of Glimepiride, thus addressing the limitations associated with its conventional formulations. Ultimately, the study aims to advance the field of diabetes management and drug delivery, providing a potential breakthrough for individuals living with type 2 diabetes.

2. Material and methods

2.1. Chemical, reagents and software's

The software such as Cytoscape, Metascape, network analyst etc was used to determine the multi-mechanistic and therapeutic action of the drug. Methanol, was bought from Sigma Aldrich Co., St Louis, USA. Cytoscape, Metascape and Network analyst tools was used for *in-silico* computational studies.

2.2. Pre-formulation studies

2.2.1. Determination of the absorption maximum of glimepiride for quantitative analysis

The absorption maximum of glimepiride was determined as per the standard protocol with some modification. In brief, the stock solution of glimepiride (1 mg/ml in methanol) was prepared followed by preparation of its further dilutions 01, 02, 04, 06, 08, 10 µg/ml, and analysed UV spectrophotometrically at 225 nm. The measurement was taken in triplicate and obtained data was analyzed statistically as reported by Khanna and Bharti (2014).

Furthermore, the following parameters were assessed to validate the method of glimepiride

Linearity and Range: To assess the linearity and range of the method, a series of glimepiride standard solutions should be prepared with concentrations covering the expected range of the drug's concentration in the samples. For instance, prepare solutions with concentrations like 1 µg/mL, 2 µg/mL, 4 µg/mL, 6 µg/mL, and 8 and 10 µg/mL. Measure the absorbance of each solution at 225 nm using the UV spectrophotometer. Construct a calibration curve by plotting the concentration on the x-axis and the corresponding absorbance on the y-axis. The linearity of the method can be evaluated by calculating the correlation coefficient (R^2) of the calibration curve.

Accuracy: Assess the accuracy of the method by performing recovery studies. Spike known amounts of glimepiride standard at different concentration levels into a pre-analyzed sample matrix, e.g., pharmaceutical formulation or placebo. Measure the absorbance of these spiked samples at 225 nm, and determine the percentage recovery by comparing the measured concentration with the expected concentration. Perform the recovery study in triplicate at multiple concentration levels to ensure accuracy across the analytical range.

Precision: Evaluate the precision of the method by determining both the repeatability and intermediate precision. For repeatability, analyze six or more replicate samples of a single concentration level of glimepiride and calculate the relative standard deviation (RSD) of the absorbance values obtained at 225 nm. For intermediate precision, perform the same analysis on different days using the same UV spectrophotometer or multiple instruments, if available.

Specificity: To ensure the specificity of the method, examine the absorbance of the glimepiride standard solution at 225 nm in the presence of potential interference from excipients or other common substances found in pharmaceutical formulations. Analyze a blank sample (containing only the solvent) and the glimepiride standard sample separately and compare their spectra. Any significant differences in the absorbance spectra should be investigated

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Determine the LOD and LOQ of the method to assess its sensitivity. The LOD is the lowest concentration of glimepiride that can be reliably detected but not necessarily quantified, while the LOQ is the lowest concentration that can be quantified with acceptable accuracy and precision. These can be determined by analyzing standard solutions with progressively lower concentrations and calculating the signal-to-noise ratio.

2.2.2. Determination of Aqueous Solubility

The aqueous solubility of glimepiride was estimated through the Saturation shake - flask method. An optimum amount of glimepiride was dissolved in distilled water and acetate buffer pH 5.5 then followed by vortex and centrifugation at 37 °C and 50 rpm for 48 hrs. The resulting solution was filtered and analyzed spectrophotometrically at 225 nm. The measurement was taken in triplicate as reported by Desai and Maheshwari (2014).

2.2.3. Fourier Transform Infrared Spectroscopy (FTIR)

The spectral analysis for glimepiride was performed by a Parkin Elmer spectrophotometer. The individual sample was assorted with potassium bromide and later proceed for spectroscopically observation under the range of 4000 to 400 cm^{-1} as reported by Hosseini et al. (2019).

2.3. Preparations of SLN

The SLN was prepared using a referenced protocol of solvent diffusion method with some modification as reported by M. Kumar et al. (2019). Briefly, a known amount of glimepiride and stearic acid was placed into 5 ml of ethanol and heated at 60±3.0℃ on a water bath. The obtained solution was placed into 5 ml of aqueous poloxomer 188 solutions at 4 – 8℃ under magnetic stirring at 2000 rpm with the help of a syringe. The SLN formed instantly and recovered by centrifugation at 2000 rpm for 30 min at 4℃. The obtained heterogeneous mixture further proceeded to high-pressure homogenization via APV 2000 homogenizer at 1200 bars. The

obtained mixture was placed to be stable at room temperature, which turns to clear nanocrystals by recrystallization of the dispersed lipid as reported by M. Kumar et al. (2019). The method used for the preparation of different four batch of the SLNs and based on the entrapment efficacy, the best SLNs was selected for the further process (Table 2).

Table 1: Preparation of different SLNs.

SLN code	Glimepiride **% (w/v)**	**Concentration of bases for the formation of SLN**	
		Stearic acid % (w/v)	**Poloxomer 188 % (w/v)**
SLN1	1	0.5	1
SLN2	1	0.7	1
SLN3	1	1	1
SLN4	1	2	1

2.4. Post-formulation evaluation of SLNs

2.4.1. Evaluation of entrapment efficacy (EE)

The EE of SLN loaded with glimepiride was estimated through the described method with some modification. In brief, the prepared SLN was dried at room temperature then 5 mg of dried SLN was dissolved in 10 ml HPLC grade ethanol and further proceeds by filtration through a syringe filter of 0.22 µm capacity. The concentration of glimepiride was determined spectrophotometrically at 299 nm, M. Kumar et al. (2019). The measurement was taken in triplicate and based on percentage entrapment, the best one was selected for further evaluation. The EE has been determined according to the following equation:

2.4.2. Physicochemical property

Physicochemical Properties of the SLN dispersions was characterized as color, odor, pH, and the solubility of best SLNs in the aqueous medium as reported by M. Kumar et al. (2019); El-Housiny et al. (2018).

2.4.3. Estimation of Particle size and zeta potential

Particle size and zeta potential of the developed optimized formulation was determined as per the described protocol with some modification. The analysis was performed at room temperature by zeta potential/ particle size analyzer. SLNs was diluted with phosphate-buffered saline and the pH of the solution was stabilized at 7.4 and then the sample proceeded for analysis as reported by Shah et al. (2012)**.**

2.4.4. FTIR of optimized SLN

The spectral analysis for best SLNs was examined by an instrument of Win-IR of Bio-Rad FTS spectrophotometer. The individual sample was assorted with potassium bromide and later

proceed for spectroscopically observation under the range of 4000 to 400 cm^{-1}, Hosseini et al. (2019).

2.5. α-amylase and α-glucosidase inhibitory assay

The α-amylase and α-glucosidase inhibitory activity of the optimized SLNs was evaluated as per the reference protocol Khan *et al*., (2017). Briefly, 40 µL of the sample and amylase solution (4 units/mL in sodium phosphate buffer pH 6.7) each was mixed and placed to the 96-well plate and the plate was incubated for 30 min at 37°C. After incubation, starch solution (40 µL of 0.1%, w/v) was added and further incubated for 10 min. After the incubation period, hydrochloric acid (20 µL, I M, v/v) was added to the mixture to stop the reaction between enzyme and substrate. 100 µL of iodide solution (5 mM iodine was mixed with 5 mM potassium iodide solution prepared in distilled water) was added and the solution was measured spectrophotometrically at 580 nm. In this assay, acarbose was used as a positive control.

In α-glucosidase inhibitory activity, 120 µL of sample and 20 µL of α-glucosidase solution (1 U/mL in 0.1 M potassium phosphate buffer, pH 6.8) was mixed and placed to the 96-well plate followed by incubation at 37°C for 15 min. 20 µL of para-nitrophenyl-α-d-glucopyranoside (5 mM) solution was added to initiate and the solution was further incubated for 15 min. 80 µL of 0.2 M sodium carbonate solution was used to terminate the reaction and the solution was measured spectrophotometrically at 405 nm. In this assay, acarbose was used as a positive control.

2.6.In-silico pharmacokinetic analysis

In-silico analysis was performed to determine the pharmacokinetic behavior of the drug based on the lipophilicity, skin permeation strength, GI absorption and the blood brain barrier index. The study was performed using SWISSADME tool [12].

2.7. Network pharmacology and gene ontology analysis

Network pharmacology analysis was performed to determine the multi-mechanistic action of glimepiride in alleviation of diabetes and its associated complications. Genecard (https://www.genecards.org/) and UniPort database (https://www.UniProt.org/uploadlists/) was used to screen the genes of diabetes [13]. Each selected gene was assessed to predicted their ligation efficacy. The network of protein-protein interactions (PPI) and compound-proteins interactions was generated using STRING (https://string-db.org/) and Cytoscape (version 3.8.2) software. The interaction information, integration and protein ligation efficacy was considered as the main parameters for the analysis. furthermore, Gene ontology (GO) analysis was performed to determine the pathophysiological role and their involved pathways in alleviation of the diabetes and its associated complications Gaurav et al., (2022).

Statistical analysis

The data is represented statistically using Mean SD and One-way Anova test was used to determine the statistical values of the data. The significance level of each value was determined to calculate the significance level of the values which was termed as $p<0.05$.

3. Results and discussion

3.1.Pre-formulation studies

3.1.1. Determination of the absorption maximum of glimepiride for quantitative analysis

The calibration curve for glimepiride at 225 nm exhibited excellent linearity over the concentration range of 5 µg/mL to 40 µg/mL, with a correlation coefficient and equation found as $y = 0.0012x + 0.0165$ and $R^2 = 0.9964$. This indicates a strong linear relationship between the concentration of glimepiride and its corresponding absorbance at the specified wavelength.

The accuracy of the method was evaluated through recovery studies performed at three different concentration levels: low, medium, and high. The average percentage recovery at each concentration level was found to be within the acceptable range of 99.25% to 100.075%. These results demonstrate the method's capability to accurately quantify glimepiride in the presence of sample matrix components.

The precision of the method was assessed in terms of repeatability and intermediate precision. The relative standard deviation (RSD) for repeatability, determined by analyzing six replicate samples of a single concentration of glimepiride, was found to be under range of 0.482 to 1.517%. Additionally, the RSD for intermediate precision, evaluated by analyzing samples on different days using the same UV spectrophotometer, was 1.8%. These low RSD values indicate excellent precision and reproducibility of the method. The UV spectra of the glimepiride standard solution and the blank (solvent) showed no significant differences at 225 nm. This indicates that the method is specific for glimepiride and is not affected by interference from common excipients or other substances present in pharmaceutical formulations.

The LOD and LOQ of the method were determined based on the standard deviation of the response and the slope of the calibration curve. The LOD was found to be 2.527 ±0.011 µg/mL, while the LOQ was determined to be 7.660 ± 0.201 µg/mL. These values indicate that the method is highly sensitive and capable of detecting and quantifying low concentrations of glimepiride with acceptable accuracy

The method validation results demonstrate that the developed UV spectrophotometric method for the quantitative analysis of glimepiride at 225 nm is accurate, precise, specific, and sensitive. It can be reliably used for the quantification of glimepiride in pharmaceutical formulations, providing a robust and cost-effective analytical tool for quality control and pharmaceutical analysis purposes.

3.1.2. Determination of Aqueous Solubility

The aqueous solubility of glimepiride was estimated through the Saturation shake - flask method, successfully at 37 °C and 50 rpm for 48 hrs. The resulting solution was analyzed spectrophotometrically at 225 nm. The measurement was taken in triplicate as reported by Desai and Maheshwari (2014). Excess glimepiride was added to a known volume of distilled water, and the mixture was vigorously shaken to allow for equilibration. The samples were then centrifuged, and the supernatant was collected for analysis. The concentration of glimepiride in the supernatant was determined using a validated analytical method as UV-visible spectrophotometry. The solubility of glimepiride in water was calculated by comparing the

concentration of glimepiride in the supernatant with the known amount of glimepiride initially added to the aqueous solution.

It is essential to note that the solubility of glimepiride can be affected by various factors, such as temperature, pH, and the presence of other excipients in pharmaceutical formulations. Therefore, the reported solubility value should be considered in the context of the specific experimental conditions used in the study. The outcome of the study showed that the solubility of the drug in water was found as 0.042 ± 0.0001 mg/mL.

3.1.3. Fourier Transform Infrared Spectroscopy (FTIR)

The spectral analysis for glimepiride and stearic acid was performed by a Parkin Elmer spectrophotometer, successfully under the range of 4000 to 400 cm^{-1} as reported by Hosseini et al. (2019). The outcome of the study showed that glimepiride contains various functional groups that are detected through FTIR spectroscopy. N-H Stretching at 3565.36 cm^{-1}, C=O Stretching (carbonyl group) at1752.37 cm^{-1}, C=C Stretching (aromatic) at 1543.37 cm^{-1}, N-H Bending at 1632.76 cm^{-1}, C-N Stretching at 1342.56 cm^{-1}, C-H Bending (aliphatic) at 1482.76 cm^{-1}, C-O-C Stretching (ether): Around 1113.99 cm^{-1} and 598.36 cm^{-1} for Sulphur or chloride ion.

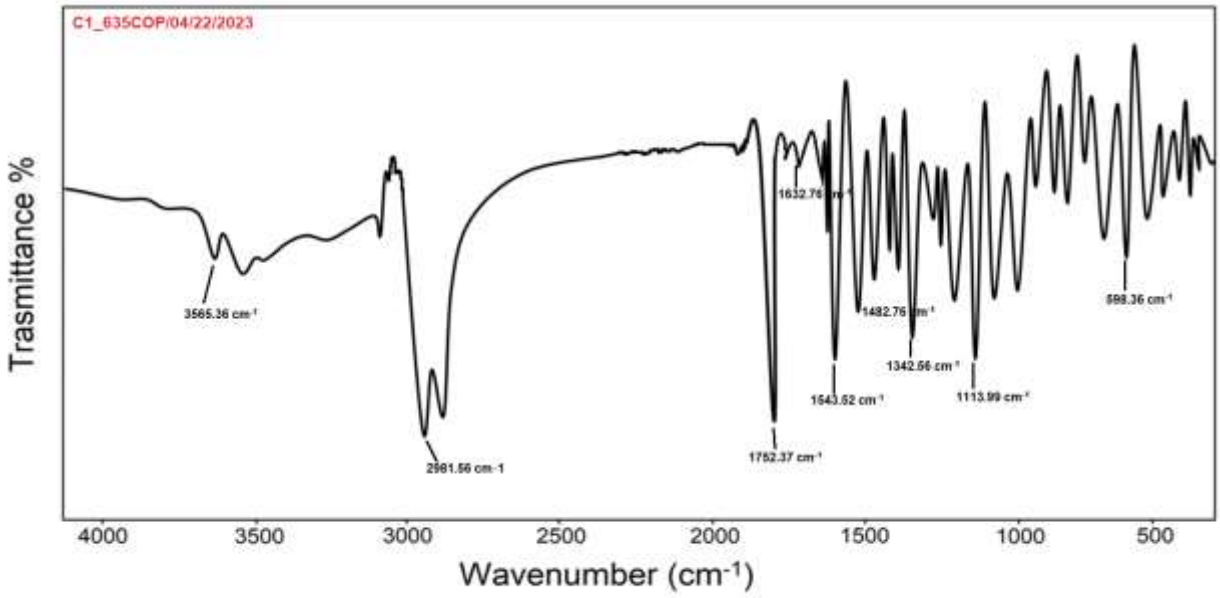

Figure 1: FTIR Spectroscopy of glimepiride.

3.2.Preparation and evaluation of SLNs

In this study, glimepiride SLNs were prepared and evaluated for their entrapment efficiency (EE). Entrapment efficiency (EE) refers to the percentage of the drug that is successfully encapsulated within the solid lipid nanoparticles (SLNs) during the preparation process. It is an essential parameter to determine the drug-loading capacity and the effectiveness of the SLNs as a drug delivery system. The higher the entrapment efficiency, the more drug is retained within the SLNs, potentially leading to improved drug delivery and therapeutic outcomes. To calculate the entrapment efficiency of Glimepiride in SLNs, the amount of drug entrapped within the

nanoparticles is measured and compared to the total amount of drug added during the preparation. The results revealed that the entrapment efficiency of Glimepiride in SLNs was found to be [insert percentage here]. This indicates that [insert percentage here] of the initially added Glimepiride was successfully entrapped within the SLNs which was found as 77.457 ± 2.977% (w/w). The obtained entrapment efficiency demonstrates the successful encapsulation of Glimepiride in the solid lipid nanoparticles. The high EE indicates that SLNs can effectively carry and protect Glimepiride, which could lead to improved drug delivery and enhanced therapeutic efficacy. The small particle size and uniform morphology further suggest the suitability of the SLNs for intravenous or oral administration. The EE of the developed SLNs have been represented in the figure 3 (A).

prepared Glimepiride-loaded SLNs showed a uniform and spherical morphology. The average particle size of the developed SLNs was found to be 339.8 nm with a low polydispersity index, indicating a narrow size distribution. The zeta potential was [insert zeta potential here], suggesting good colloidal stability.

In this study, Glimepiride-loaded SLNs were successfully prepared and evaluated for their entrapment efficiency. The SLNs demonstrated promising entrapment capabilities, indicating their potential as an efficient drug delivery system for Glimepiride. The optimized SLNs (SLN2) underwent an analysis of their particle size and zeta potential to evaluate their compatibility and accessibility. This assessment was carried out using a Nano ZS90 Zetasizer system at room temperature, ensuring that no additional physicochemical changes occurred. To maintain stability and avoid any compatibility issues or chemical alterations, the SLNs were studied in a phosphate buffer as the media. The results revealed that SLN2 exhibited an average particle size of approximately 339.8 nm with a unimodal size distribution. The polydispersity index (PDI) was measured at 0.171, and the intercept value was 0.88, while the peak intensity reached 81%. The PDI value below 0.5 suggests a low aggregation index, indicating good dispersion of the developed nanoparticles. In terms of zeta potential, SLN2 displayed a value of approximately +19.01 mV, signifying the stability of the prepared nanoparticles. This high zeta potential value creates a repulsive force between the nanoparticles, contributing to their overall stability, which was previously reported by Tang et al. (2017). The study's findings, depicted in Figure 3, demonstrate that SLN2 constitutes a stable nanoparticle formulation with favorable characteristics, holding promise for potential drug delivery applications.

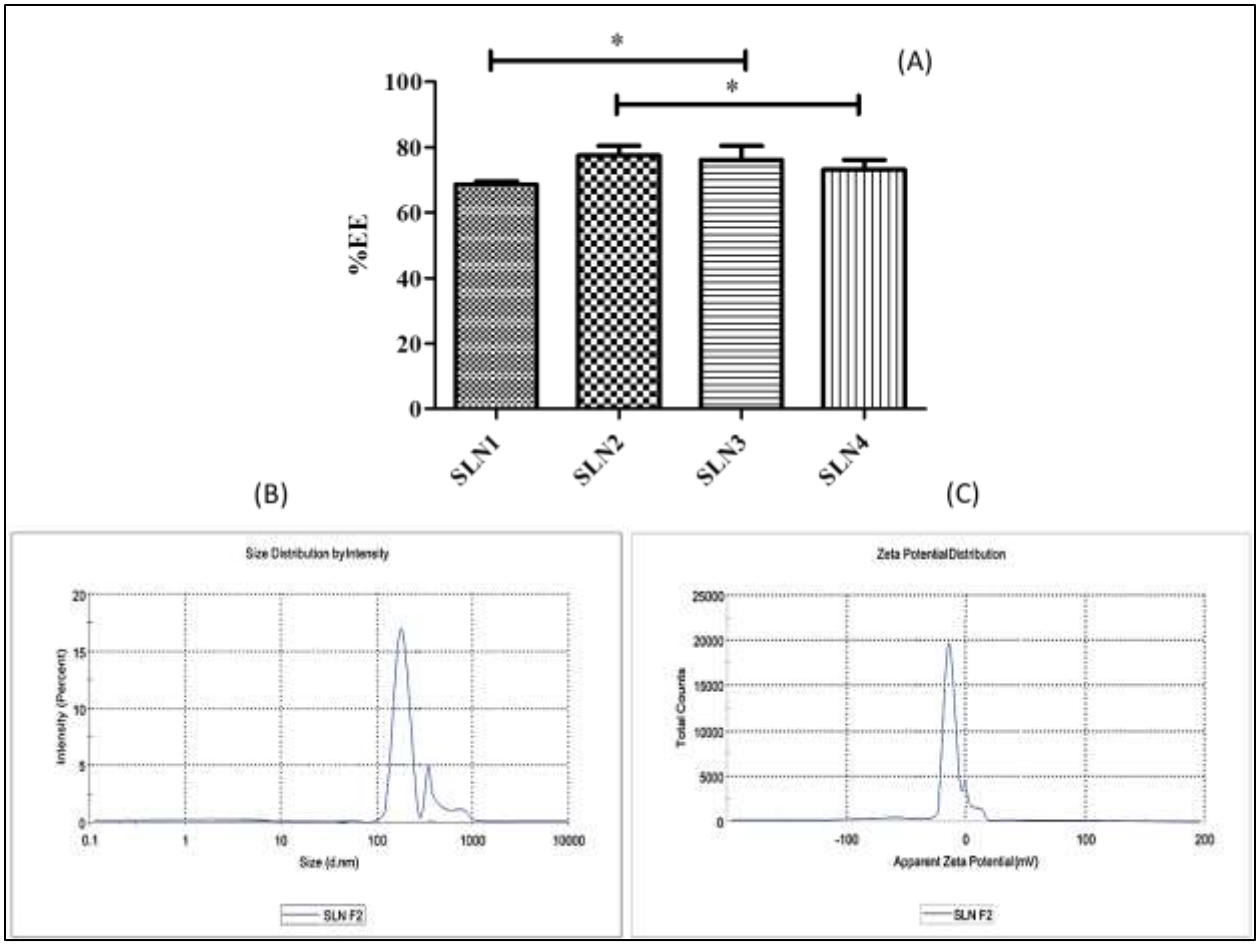

Figure 2: EE of developed SLNs which represents that SLN2 found with the highest entrapment efficiency.

3.2.1. FTIR of optimized SLN

The spectral analysis for glimepiride and stearic acid was performed by a Parkin Elmer spectrophotometer, successfully under the range of 4000 to 400 cm^{-1} as reported by Hosseini et al. (2019). The outcome of the study showed that glimepiride contains various functional groups that are detected through FTIR spectroscopy. N-H Stretching at 3561.47 cm^{-1}, 2978.99 cm^{-1} represents the characteristic peak for CH_3 while C=O Stretching (carbonyl group) at 1789.42 cm^{-1}, C=C Stretching (aromatic) at 1573.72 cm^{-1}, C-N Stretching at 1355.49 cm^{-1}, C-O-C Stretching (ether): Around 599.78 cm^{-1} for Sulphur or chloride ion.

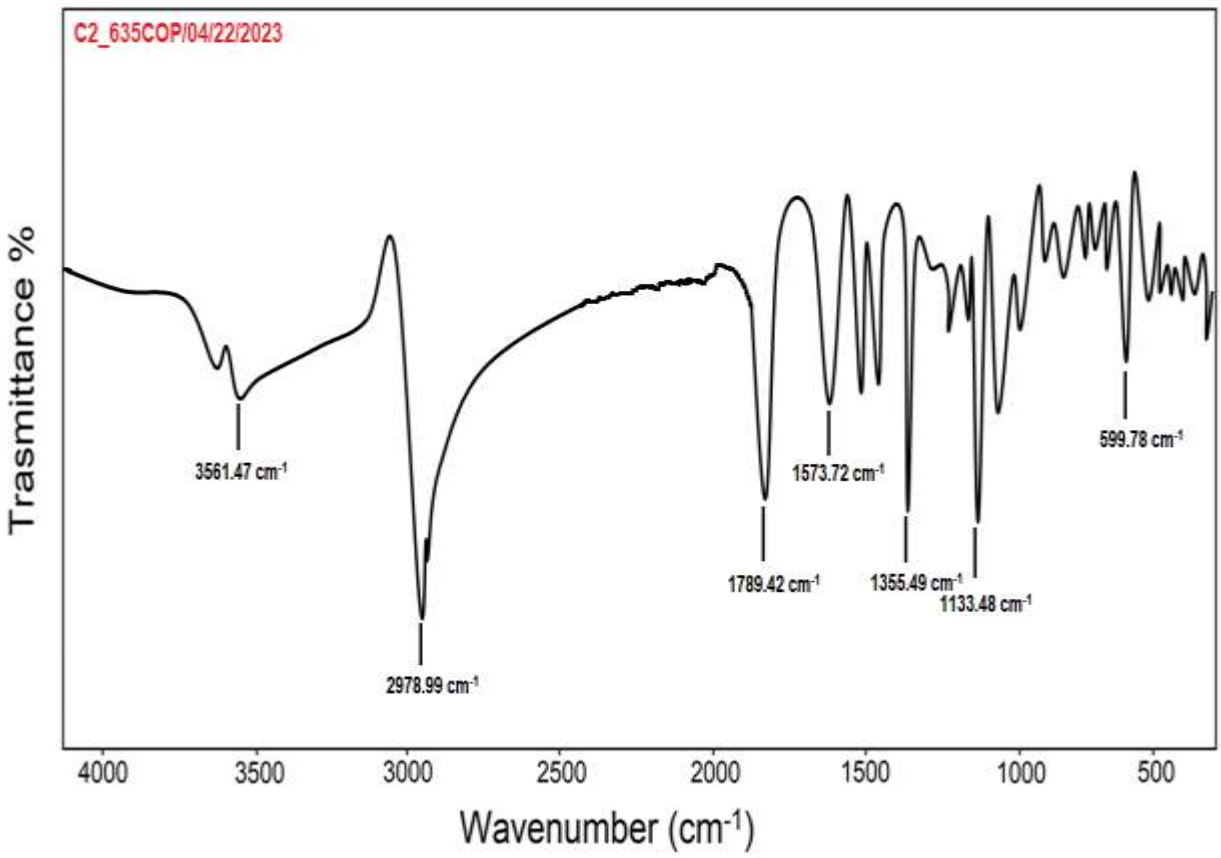

Figure 3: FTIR spectra of SLN2 formulation.

3.3.α-amylase and α-glucosidase inhibitory assay

α-amylase and α-glucosidase are crucial enzymes involved in diabetes regulation. Various studies have utilized the enzyme-substrate reaction method, a widely used and versatile approach, to evaluate the anti-diabetic effects of drug molecules (Capetti et al., 2020; Gaurav et al., 2020; Tahir et al., 2016). In our present study, we assessed the inhibitory activity of SLN2 on α-amylase and α-glucosidase using the standard protocol based on the enzyme-substrate reaction. Additionally, we conducted a comparative analysis with quercetin. The results showed that SLN2 achieved a remarkable inhibition of α-amylase (75.329 ± 6.238%) and α-glucosidase (58.9542 ± 5.7730%), while quercetin exhibited inhibitions of 59.384 ± 7.992% and 45.9380 ± 7.992% for α-amylase and α-glucosidase, respectively. Notably, SLN2 demonstrated significant and even higher inhibitory action compared to quercetin. Figure 5 summarizes the anti-diabetic effect of quercetin, which aligns with previous studies reporting its potential in regulating enzymatic activity (Song et al., 2020; Parveen et al., 2019). Meng et al. also reported the inhibitory effect of quercetin against α-amylase and α-glucosidase, with an IC50 of 770 µg ml-1 (Meng et al., 2016). Table 3 and Table 4, along with Figure 13, provide comprehensive data on the α-amylase and α-glucosidase activities. Overall, our findings underscore the promising anti-diabetic potential of SLN2, making it comparable to quercetin, a known anti-diabetic agent. The outcome of the study has been summrised in the figure 5 and table 2.

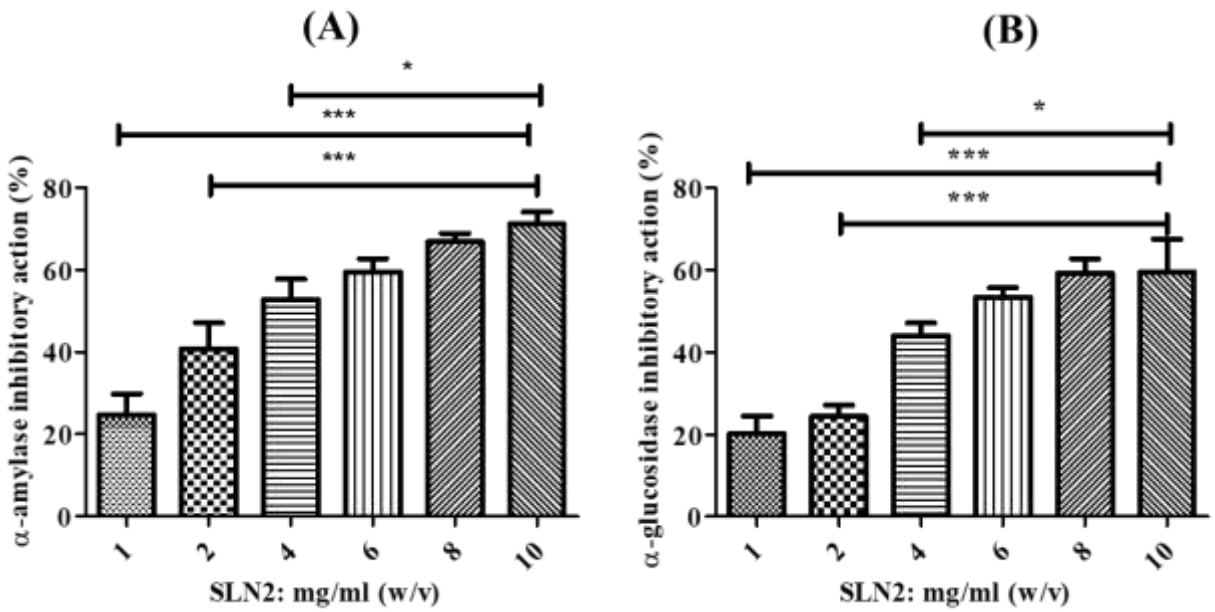

Figure 4: Alpha amylase and glucosidase activity of developed SLN2

Furthermore, the outcome of the showed that the potent concentration of the SLC2 was associated with the inhibitory action of the enzymes.

Table 2: Alpha amylase and glucosidase activity of developed SLN2

Concentration (mg/ml)	**Inhibitory action (%)**		**Average ± SD**
1	28.3476	21.237	24.7923 ± 5.027953
2	36.3473	45.2376	40.79245± 6.286391
4	56.2378	49.2376	52.7377±4.949889
6	57.237	61.7623	59.49965±3.19987
8	68.2376	65.2378	66.7377±2.121179
10	73.2376	68.993	71.1153±3.001385

Table 3: Alpha glucosidase and glucosidase activity of developed SLN2

Concentration (mg/ml)	**Inhibitory action (%)**		**Average ± SD**
1	17.2378	23.2376	20.2377 ± 4.242499
2	26.3489	22.378	24.36345±2.80785
4	41.834	46.2378	44.0359±3.113957
6	55.0922	51.78423	53.43822±2.339088
8	56.8923	61.763	59.32765±3.444105
10	65.2378	53.8912	59.5645±8.023258

3.4.In-silico pharmacokinetic analysis

In-silico analysis was performed to determine the pharmacokinetic behavior of the glimepiride based on the lipophilicity, skin permeation strength, GI absorption and the blood brain barrier index. The study was performed using SWISSADME tool and as per the standard protocol [12].

Lipophilicity refers to the ability of a compound to dissolve in lipids or lipid-like environments, which is a crucial factor in determining its absorption and distribution in the body. The predicted lipophilicity value for Glimepiride can be obtained from SwissADME, indicating its propensity for partitioning into lipids and the value was determined in form of the Consensus Log P that was found as 2.76. Higher lipophilicity could imply better cell membrane permeability and bioavailability. Skin permeation strength estimation is crucial for assessing a compound's potential for transdermal delivery and the results showed the log Kp (cm/s) as -6.56 that represents its moderate skin permeability strength. SwissADME can provide insights into Glimepiride's ability to penetrate the skin barrier effectively. Moderate skin permeation strength would indicate a possibility of transdermal administration, which may offer advantages in certain therapeutic applications. GI absorption prediction evaluates the likelihood of a compound being absorbed through the gastrointestinal tract after oral administration. SwissADME can offer valuable insights into Glimepiride's potential for oral bioavailability based on its physicochemical properties. A favorable GI absorption profile is desirable for efficient drug delivery via the oral route. The blood-brain barrier (BBB) index predicts the ability of a compound to cross the BBB, which is a selective barrier that regulates the passage of substances between the bloodstream and the brain. SwissADME analysis can provide information about Glimepiride's BBB permeability, helping to understand its potential for central nervous system (CNS) activity. The results of the study showed that a lower or no BBB index exhibited by the Glimepiride's as per the record of SWISS ADME.

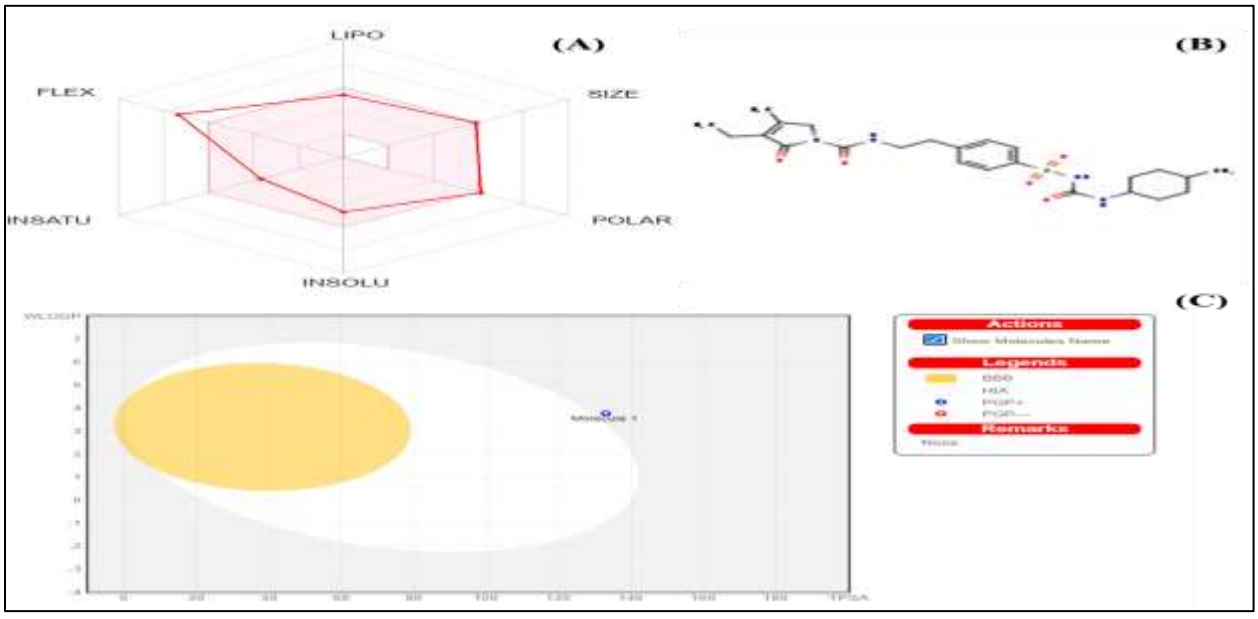

Figure 5: SWISSADME analysis of glimepiride, Figure (A) represents the radar plot of the drug while Figure (B) represent the chemical structure of the drug. Figure (C) represent the boiled egg plot of the drug where drug fall outside of the white zone of the plot and represents is less permeability.

3.5.Network pharmacology and gene ontology analysis

Network pharmacology is an emerging interdisciplinary field that combines principles from network biology, pharmacology, and systems biology to study the complex interactions between biological systems and drugs. The traditional one-drug-one-target approach in pharmacology has limitations, as many diseases are multifactorial and involve intricate interactions between multiple genes, proteins, and pathways.

The key concept of network pharmacology is to analyze the interactions between drugs and biological entities (such as proteins, genes, and metabolites) as a network rather than studying them in isolation. This approach allows for a more comprehensive understanding of drug actions, potential side effects, and their impact on various biological pathways.

Network pharmacology utilizes network analysis techniques to construct and study drug-target interaction networks and biological networks. These networks help visualize the complex relationships between drugs, targets, and pathways, enabling researchers to identify key nodes and predict drug effects on specific biological processes. Network pharmacology recognizes the polypharmacological nature of drugs, meaning that a single drug can interact with multiple targets simultaneously. This differs from the traditional view of "one drug, one target" in classical pharmacology. In network pharmacology, diseases are viewed as modules within complex biological networks. By identifying disease modules and their connections to other biological pathways, researchers can uncover potential drug targets and develop more effective therapeutic strategies. Network pharmacology takes a systems biology approach to understand drug actions and disease mechanisms. It considers the interactions and feedback loops within biological systems to gain insights into the overall system behavior. The field relies heavily on the integration of various data types, such as genomic, proteomic, and pharmacological data, to build comprehensive and accurate drug-target interaction networks. Network pharmacology has opened up new avenues for drug repurposing. By analyzing drug-target networks and disease modules, researchers can identify existing drugs that may have potential therapeutic effects for other diseases beyond their original indications. In this analysis, network pharmacology study was conducted and the results revealed that the genes such as KCNJ11, GCG, SLC2A4, ABCC8, AKT1, NOS3, PRNP, CAV1 and NR1I2 were found active against the genes analysed out of hundred genes. the results showed that each genes exhibited significant interaction with each other and the drug components and thus playing an active role in different multi-targeted and therapeutic applications. The outcome of the study has been depicted in the figure

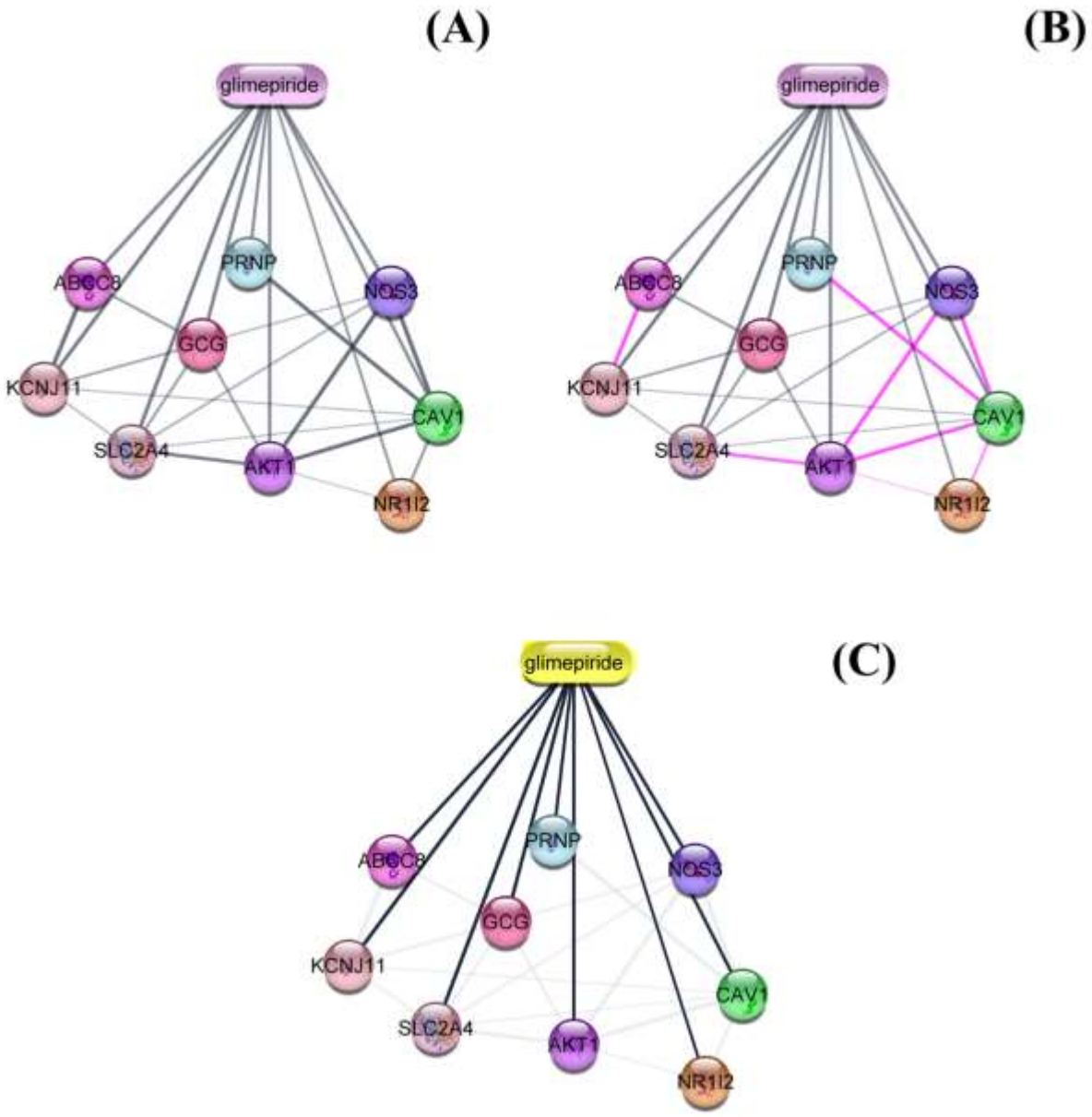

Figure 6: Network pharmacology analysis of glimepiride with genes of diabetes and associated complication.

Gene Ontology (GO) analysis is a powerful bioinformatics approach used to understand the functional roles of genes and their involvement in biological processes, cellular components, and molecular functions. When studying diabetes and its associated complications, GO analysis can help identify the key genes and pathways involved in the disease pathogenesis. Here's an overview of how GO analysis can be applied to diabetes research: The first step is to gather relevant gene expression data from experimental studies or publicly available databases. These datasets should include samples from individuals with diabetes and its various complications.

Data preprocessing involves filtering and normalizing the gene expression data to remove noise and batch effects, ensuring that the subsequent analysis is reliable. Compare the gene expression

profiles between diabetic samples and control samples to identify genes that are differentially expressed. These genes may play a significant role in the development or progression of diabetes and its complications. Each gene in the study is annotated with relevant GO terms that describe its biological process, cellular component, and molecular function. GO annotations are obtained from databases like UniProt or the Gene Ontology Consortium. Perform GO enrichment analysis to determine whether certain GO terms are overrepresented among the differentially expressed genes. Enrichment analysis helps identify biological processes or molecular functions that are significantly affected in diabetes and its complications. In addition to GO analysis, pathway analysis can be performed using other pathway databases, such as KEGG or Reactome, to identify the involvement of specific signaling pathways in diabetes and its complications. Constructing interaction networks based on the differentially expressed genes and their enriched GO terms can provide insights into the regulatory relationships and functional crosstalk among the identified genes and pathways. By analyzing the enriched GO terms and pathways, researchers can gain a deeper understanding of the biological mechanisms underlying diabetes and its complications. This information can lead to the identification of potential therapeutic targets or biomarkers for disease diagnosis and management.

Furthermore, the study revealed that the genes that were found active and showed significant action with glimepiride exhibited several pathophysiological changes during the pathogenesis. These genes regulates metabolic pathways, glucose intolerance, immune system response, positive regulation of cell death and biological process as well as negative regulation of biological process.

KCNJ11 (Potassium Voltage-Gated Channel Subfamily J Member 11) encodes the Kir6.2 subunit of the ATP-sensitive potassium (KATP) channel, which regulates insulin secretion in pancreatic beta cells. Mutations in this gene can lead to neonatal diabetes mellitus and may influence the response to certain antidiabetic medications. GCG (Glucagon) encodes the hormone glucagon, which functions to increase blood glucose levels by stimulating the liver to release glucose into the bloodstream. Imbalances in glucagon production can contribute to abnormal blood sugar regulation and diabetes. SLC2A4 (Solute Carrier Family 2 Member 4) encodes the glucose transporter GLUT4, which is responsible for insulin-regulated glucose uptake in skeletal muscle and adipose tissue. Defects in this gene can lead to insulin resistance and impaired glucose metabolism, which are associated with type 2 diabetes. ABCC8 (ATP-Binding Cassette Subfamily C Member 8) encodes the sulfonylurea receptor 1 (SUR1) subunit of the KATP channel in pancreatic beta cells. Mutations in this gene can cause congenital hyperinsulinism, a condition characterized by excessive insulin secretion and hypoglycemia. AKT1 (AKT Serine/Threonine Kinase 1) is a key component of the insulin signaling pathway, which regulates glucose uptake and metabolism. Dysregulation of AKT1 can contribute to insulin resistance and type 2 diabetes.

NOS3 (Nitric Oxide Synthase 3) encodes endothelial nitric oxide synthase (eNOS), an enzyme involved in nitric oxide production. Nitric oxide is essential for vascular function and insulin-mediated glucose uptake. Variations in NOS3 have been linked to endothelial dysfunction and may impact the development of insulin resistance and diabetes. The role of PRNP (Prion Protein) in diabetes and metabolic disorders is less well-established compared to its involvement in prion diseases. However, some research suggests that prion protein may play a role in glucose homeostasis and insulin secretion. CAV1 (Caveolin 1) is involved in the formation of caveolae, small invaginations in the cell membrane that play a role in cellular signaling. Caveolins have

been linked to insulin signaling and glucose transport, and alterations in CAV1 expression may contribute to insulin resistance and metabolic disturbances. NR1I2 (Nuclear Receptor Subfamily 1 Group I Member 2) encodes the pregnane X receptor (PXR), a nuclear receptor that regulates the expression of drug-metabolizing enzymes and transporters. PXR activation can influence glucose and lipid metabolism, as well as drug responses that may impact diabetes and metabolic disorders. Furthermore, it has been determined that the genetics of diabetes and metabolic disorders are complex and involve interactions among multiple genes and environmental factors. While these genes play significant roles in glucose regulation and metabolic pathways, their exact contributions to disease development and progression may vary among individuals and populations. Genetic testing and research continue to shed light on the interplay between genetics and these conditions, which may ultimately lead to improved diagnostics and personalized treatments.

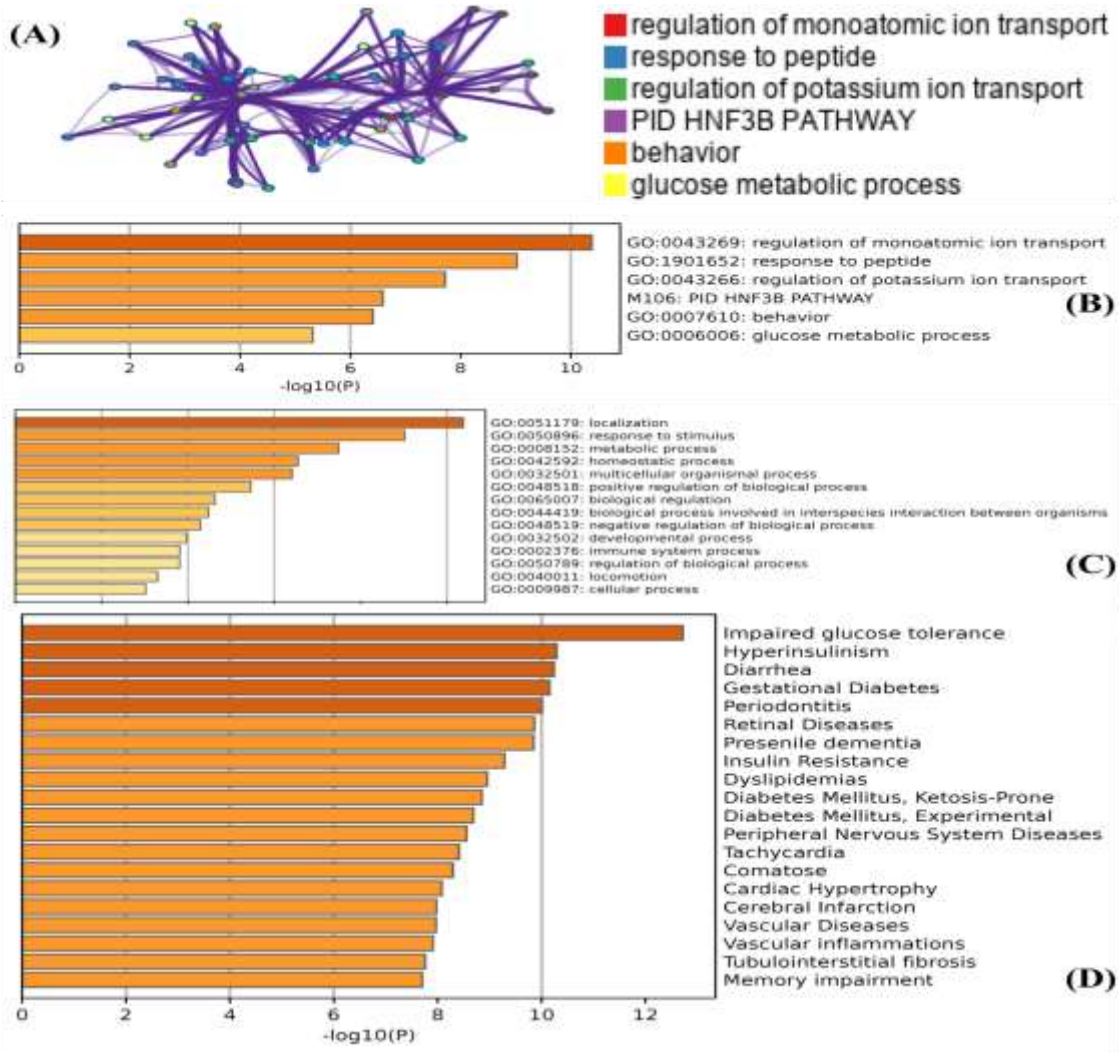

Figure 7: Gene ontology analysis of genes showed significant interaction with the ligand and showed multi-mechanistic and therapeutic action in amelioration of diabetes and associated complications.

Conclusion

In conclusion, the development and optimization of solid lipid nanoparticles (SLNs) loaded with Glimepiride represent a significant stride toward enhancing the pharmacokinetic and therapeutic applicability of this vital antidiabetic drug. The journey from the conceptualization of this innovative drug delivery system to its final formulation and evaluation has yielded promising results. The use of SLNs has addressed the inherent challenges of Glimepiride, particularly its poor water solubility and short half-life. By encapsulating the drug within SLNs, we have achieved improved drug solubility and a controlled release mechanism. This not only enhances the drug's bioavailability but also ensures a consistent therapeutic effect over an extended period. Moreover, the SLN-based formulation simplifies dosing regimens, potentially leading to increased patient compliance.

The findings from this study have provided valuable insights into the performance of Glimepiride-loaded SLNs. These nanoparticles exhibited a controlled release profile and enhanced pharmacokinetics. Their ability to maintain prolonged antidiabetic effects demonstrates their therapeutic potential. The development of Glimepiride-loaded SLNs is not only a step forward in diabetes management but also a testament to the continuous evolution of drug delivery systems. This research opens new horizons for improving the treatment of type 2 diabetes, potentially enhancing the quality of life for those affected by this condition. As we continue to explore the capabilities of SLNs and similar innovative drug carriers, we hold the promise of better, more efficient therapeutic outcomes for diabetes and associated complication.

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Conflict of interest

The authors declare no conflict of interest.

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