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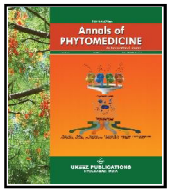

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Antidiabetic potential of developed solid lipid nanoparticles loaded with quercetin: *In vitro* and *in silico* studies

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Abstract

Development of the novel formulation is critically needed for alleviating several acute and chronic ailments. Diabetes is acknowledged as one of the prevalent metabolic disorder affecting the global health and economy, vigorously. Based on the above facts, the study is aimed to develop SLNs (solid lipid nanoparticle) loaded with quercetin as the potential novel formulation for the management of diabetes. Preformulation and postformulation studies were conducted to determine the purity and compatibility of quercetin with the formulation bases used for its development. a-amylase and a-glucosidase inhibitory activity and DPPH (2, 2-diphenyl-1-picrylhydrazy) free radicals scavenging activity were performed of the developed formulation with the comparison of the control group quercetin to determine antidiabetic and antioxidant potential. Network pharmacology analysis was performed to determine the multimechanistic and therapeutic potential of quercetin for treatment of diabetes and its associated complications. The results showed that quercetin was found substantially compatible with the formulation bases used for its development. The particle size of the developed SLNs was found with no drug interaction with the formulation base which was evaluated from the FTIR (Fourier transform infrared spectroscopy) analysis. In antidiabetic analysis of the formulation, no significant (p<0.05) variance was found in the a-amylase and a-glucosidase activity while DPPH activity of the developed SLNs was found 81% at the higher concentration. In network pharmacology analysis, it was found that quercetin regulates several genomes such as IRS-1, TLRs, ILs, CASPs, MAPKs, AKT1, AOX, *etc*., involved in the diabetes and its associated complication. Hence, it can be concluded that the developed SLNs not only regulates diabetes even effect for the diabetes associated complication.

1. Introduction

Diabetes mellitus has been acknowledged as the chronic metabolic disease and characterized by least production or application of insulin. Hyperglycemia is symptomized to unrestrained diabetes, and over time, it causes several organ and physiological morbidities (Khan, and Zahiruddin, 2020). In diabetes generally, T2DM (Type 2 diabetes mellitus) has been characterized as the metabolic disorders of endocrine system, where inadequate insulin excretion and its confrontation are the basic T2DM characteristics (Mohan *et al*., 2012). Although, a-amylase and a-glucosidase enzymes have been acknowledged as the digestive enzymes which play an essential role in digestion as well as absorption of the glucose in hyperglycemia. The a-amylase enzyme breakdown/hydrolyzes polysaccharides from their alpha linkage and a-glucosidase controls postprandial hyperglycemia. Antioxidants play an essential role in suppression of the oxidative stress induced by the varieties of the ketone bodies and excessive metabolic toxins while a simultaneous therapy a-amylase and a-glucosidase inhibitors offers an additive effect in alleviation of diabetes *via* reducing high blood glucose level (Van Quan *et al*., 2019).

WHO (World health organization) report revealed that diabetes would be the 7th leading cause of morbidity and mortality by 2030. Furthermore, it has been reported that about 1.5 million deaths has been done due to the diabetes in 2012 and it was found more abundant in female as compared to the male (WHO, 2016). IDF 2019 (International Diabetes Federation), 9th edition report published that diabetes is the most exponentially growing health emergencies in 21st century throughout the globe. In a reported published by IDF in 2019, it was reported that about 463 million people are associated with the diabetes which would be triggered to many folds by 2045 (Nam Han Cho *et al*., 2017).

The modern system of medicine has been acknowledged as the most effective and credible medicinal system for treating various acute and chronic ailments. Many of the oral hypoglycemic drugs have been found; namely, biguanides, sulfonylureas, thiazolidinediones, a-glucosidase inhibitors and non-sulfonylureas secretagogues for the treatment of the diabetes. Although, with the tremendous use and maintaining the system for the well-being of the humans against various pathophysiological onsets, the perfection is still far away to reach out the effective, affordable and accessible therapy for the treatment of the diabetes. Because of various limitations associated with the modern medicines; namely, adverse effects, drug resistance,

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and toxicity prompts the healthcare professionals for development of newer, effective and affordable antidiabetic pharmaceutical from natural sources (Salehi *et al*., 2019; Ali *et al.*, 2022).

Solid lipid nanoparticles (SLN) are an advanced pharmaceutical novel drug delivery system (NDDS) in the modern era of pharmaceuticals. SLN was discovered in 1991, which represents traditional colloidal carriers such as polymeric and microliposomes emulsions and nanoparticles The conventional approach of SLN is associated to enhance drug permeation ability, good release profile and targeted drug delivery with excellent physical stability and low degradability, *etc*. (Muller *et al*., 2000; Naseri *et al*., 2015; Verma *et al*., 2021). Nanoparticles ranging from 10 to 1000 nm in size have promising effects in enhancing the bioavailability of the drug. The formulations hyphenated with SLN is a major consideration in the era of colloidal drug carrier system which generates an alternative particulate in the field of NDDS (Mukherjee *et al*., 2009; Kumar *et al.*, 2020).

Natural products are one of the enriched sources for new drug discovery and development as they are associated with the multidimensionality of the constituents. There are several varieties of the phytoconstituents which play an important role in mitigating various ailments. The major class of constituents present in the natural products are; namely, phenols, flavonoids, alkaloids, glycosides, *etc*., despite the multimechanistic property of natural products or their constituents, quality based evaluation is also needed for their regulatory purpose.

Fourier transform infrared (FTIR) and ultra violet visible spectroscopy are the most important, economic and sophisticated techniques which make us far easier in quality and quantity based evaluation of active pharmaceutical ingredients.These techniques are highly associated in forensics laboratory, research laboratory for the analysis of the blood samples and characterization of the unknown samples (Bram and Wolfram, 2017).

For decades, computational approaches have been playing an immense role in drug design, synthesis and to evaluate the biological interaction of newly developed drug molecules. Network pharmacology is one of the computational approaches which explore the multimechanistic role of any therapeutically active drug molecule based on the multiple genomic interactions at a single time. Protein-protein interaction and protein-drug interaction explore the role of selected targets in related dysfunction and while protein-drug interaction reveals the ligation efficacy of a drug molecule based on the degree of ligation (Yi *et al*., 2018; Zhang *et al*., 2019).

Quercetin is a flavonoid compound acknowledged with the several pharmacological activities such as antioxidant, antidiabetic, hepatoprotection, nephroprotection, *etc*., despite of several reports published in various reputed scientific journals; multimechanistic role of quercetin is still a lacking part which may fulfils obsession of scientific needs. Besides, the potential therapeutic role of quercetin, development of adorable and potential pharmaceuticals which may enhance their biological potency is still a concerning need in the field of pharmaceuticals. Besides the above facts, multimechanistic role of quercetin is still needed at molecular level which would uncover the molecular mechanism of quercetin in combating the diabetes. Taking these facts into consideration and utilization of quercetin as a potential active ingredient against various diseases including diabetes, the study is aimed to develop the solid lipid nanoparticles loaded with quercetin and their evaluation for antidiabetic activity using *in vitro* approaches. The molecular mechanism of the quercetin would be evaluated using network pharmacological approach, thus validating the facts of quercetin as a potential antidiabetic agent.

2. Materials and Methods

2.1 Chemicals, reagents and software's

a-Amylase, a-glucosidase, pNPG, fast blue, 2,2-diphenyl-1-picrylhydrazyl (DPPH), were bought from Sigma Aldrich Co., St Louis, USA. Cytoscape, metascape and network analyst tools were used for *in silico* computational studies.

2.2 Preformulation studies

2.2.1 Determination of the absorption maximum of quercetin for quantitative analysis

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

2.2.2 Determination of aqueous solubility

The aqueous solubility of quercetin was estimated through the Saturation shake-flask method. An optimum amount of quercetin 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 h. The resulting solution was filtered and analyzed spectro-photometrically at 370 nm. The measurement was taken in triplicate (Desai and Maheshwari, 2014).

2.2.3 Determination of lipophilicity

Lipophilicity of quercetin was determined through the traditional shake-flask method as described in protocol with some modification. In brief, an optimum uniform amount of quercetin was poured into three different volumetric flasks and then the measured quantity of lipids such as stearic acid, prectrol, dynasan 114 placed to each flask simultaneously. The resulting erogeneous mixture proceeded to the vortex and then centrifugation at 50 rpm at 37°C for 48 h. The supernatant was collected and filtered using a syringe filter of 0.22 mm. The filtrate was then analyzed spectrophotometrically at 370 nm (Desai and Maheshwari, 2014).

The further partition coefficient of the quercetin was determined using n-octanol and water partition system. The measured amount of quercetin was placed into a conical flask containing measured volumes of an n-octanol and aqueous buffer solution. The flask was shaken with a uniform time interval for 48 h to attain equilibrium and then the resulting mixture placed to a separating flask with a final shaking and kept remains undisturbed to be separated into two layers. The targeted measurement was proceeded to be analyzed spectrophotometrically at 370 nm. The resulting values of both the phases was determined in the form of the log10P of the ratio was calculated. All the measurement was taken in triplicate (Dhama *et al*., 2022).

2.2.4 Fourier transform infrared spectroscopy (FTIR)

The spectral analysis for quercetin and stearic acid was performed by a Perkin 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} (Hosseini *et al*., 2019).

2.3 Preparations of SLN

The SLN was prepared using a referenced protocol of solvent diffusion method with some modification (Kumar *et al.*, 2019). Briefly, a known amount of quercetin and stearic acid was placed into 5 ml of ethanol and heated at 60 ± 3.0°C on a water bath. The obtained solution was placed into 5 ml of aqueous poloxomer 188 solutions at 4-8°C 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°C.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 (Kumar *et al*., 2019). The method used for the preparation of different four batches of the SLNs and based on the entrapment efficacy, the best SLNs were selected for the further process presented in Table 1.

Table 1: Preparation of different SLNs

SLN Code	Quercetin % (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 quercetin 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 mm capacity. The concentration of quercetin was determined spectrophotometrically at 299 nm (Kumar *et al*., 2019). The measurement was taken in triplicate and based on percentage entrapment, the best one was selected for further evaluation.

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 (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 (Shah *et al*., 2012).

2.4.4 FTIR of optimized SLN

The spectral analysis for best SLNs was performed by a Win-IR, 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 a-amylase and a-glucosidase inhibition assay

The a-amylase and a-glucosidase inhibitory activity of the optimized SLNs was evaluated as per the reference protocol (Khan *et al*., 2017). Briefly, 40 ml 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 ml of 0.1%, w/v) was added and further incubated for 10 min. After the incubation period, hydrochloric acid (20 ml, I M, v/v) was added to the mixture to stop the reaction between enzyme and substrate. 100 ml of iodide solution (5 mM iodine was mixed with 5mM 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 a-glucosidase inhibitory activity, 120 ml of sample and 20 al of a-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 ml of para-nitrophenyl-a-d-gluco-pyranoside (5 mM) solution was added to initiate and the solution was further incubated for 15 min. 80 ml of 0.2M 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 DPPH free radical scavenging activity

The antioxidant activity of the optimized SLNs was determined by the described protocol with some modifications (Parveen *et al*., 2019). A 20 µl of the sample and 180 ml of DPPH solution (prepared in methanol; 0.01 mM) solution was mixed and placed, in 96 well plates followed by incubation at room temperature for 30 min in a relatively dark place. After the period of incubation, the solution was measured spectrophotometrically at 517 nm. In this assay, the positive control was used as ascorbic acid.

2.7 Network pharmacology and gene ontology analysis

Network pharmacology analysis was performed to determine the multimechanistic action of quercetin in alleviation of diabetes and its associated complications. Genecard (https://www.genecards.org/) and UniPortdatabase (https://www.UniProt.org/uploadlists/) were used to screen the genes of diabetes (Li *et al*., 2021). Each selected gene was assessed to predicted their ligation efficacy. The network of protein-protein interactions (PPI) and compound-proteins interactions was generatedusing string (https://string-db.org/) and cytoscape (version 3.8.2) software. The interaction information, integration and protein ligation efficacy were 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).

2.8 Statistical analysis

The data was represented statistically as Mean ± SD, followed by Tukey test to compare multiple columns in each pair. The significance level was determined in term of the *p*-value which could not less than < 0.05.

3. Results

3.1 Preformulation studies

Pre-formulation studies were conducted to determine the compatibility and the stability of the active pharmaceutical ingredient.

For qualitative and quantitative evaluation of the quercetin, the UV absorption maxima was determined in methanol as the carrier solvent. The prepared solution of quercetin was measured spectrophotometrically at different concentrations. The outcome of the study showed that the absorption maxima of quercetin was found at the λmax of 366 nm. Furthermore, the calibration curve was plotted against the different concentrations and the obtained maxima. Each measurement was taken in triplicate for determination of the statistical difference among each measured concentration. The calibration equation for the developed method was found as y = 0.0575x + 0.0056, R^2 = 0.9936. The calibration plot and the absorption spectra of UV spectrophotometry has been depicted in Figure 1.

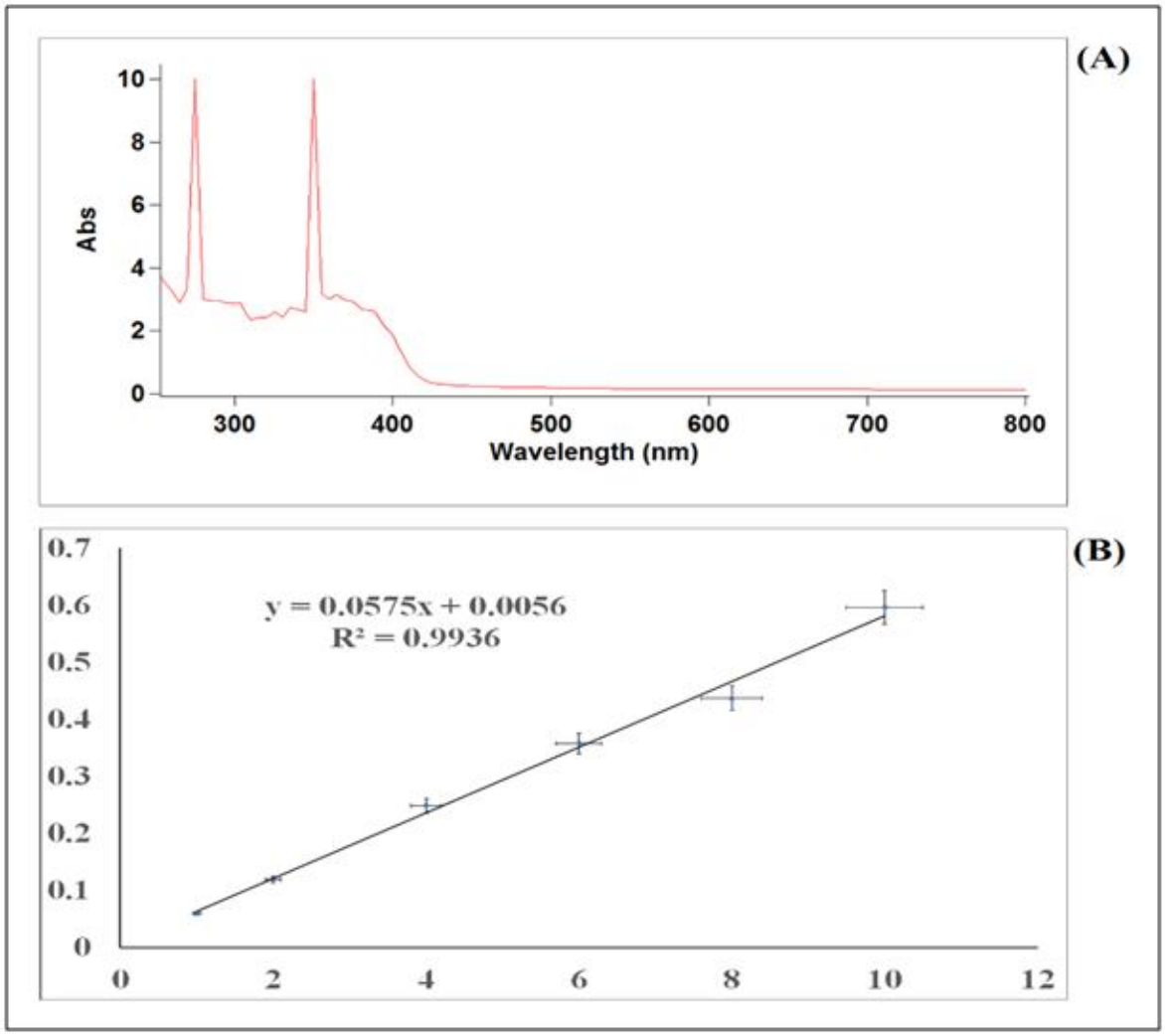

Figure 1: Absorption maxima and the calibration curve of quercetin.

The aqueous solubility and lipophilicity ratio of quercetin was determined to evaluate its solubility behavior. The results showed a significant difference in the solubility ration of quercetin in aqueous media and lipophilic media. The aqueous solubility of quercetin was found as 2.542 ± 0.298 µg/ml, while the solubility of quercetin in lipid bases such as stearic acid, prectrol, dynasan 114 was found 134.58 ± 1.992 µg/ml. FTIR analysis of quercetin was performed to determine the purity of quercetin and its originality. The results of FTIR analysis showed several principal peaks absorption at 3411.37 and 3291.01 cm^{-1} represents the stretching of -OH and CH_2 group, two moderate peaks were appeared at the frequency range 1673.73 and 1618.23 cm^{-1} represents the stretching vibration of -C=C and -C=O group while the peaks appeared at the frequency range 1207.99, 1172.69 and 827.83 cm^{-1} represents the stretching vibration of -C-O-C-, -C-H and aromatic hydrogen scissoring vibrations.

3.2 Postformulation evaluation of SLNs

Postformulation analysis of the developed formulation was determined, successfully. The parameters such as EE, physico-chemical properties, particle size and zeta potential followed by the FTIR analysis were performed. In entrapment analysis of quercetin, it was found that out of four developed SLNs, SLN2 was found as the optimistic SLNs with high percentage of drug entrapment. The EE of the drug was calculated *via* estimation of free drug in the media (Nouri *et al*., 2020). The graphical representation of EE has been depicted in Figure 2. The physiochemical properties of the optimized SLNs were found favorable as the optimistic formulation.

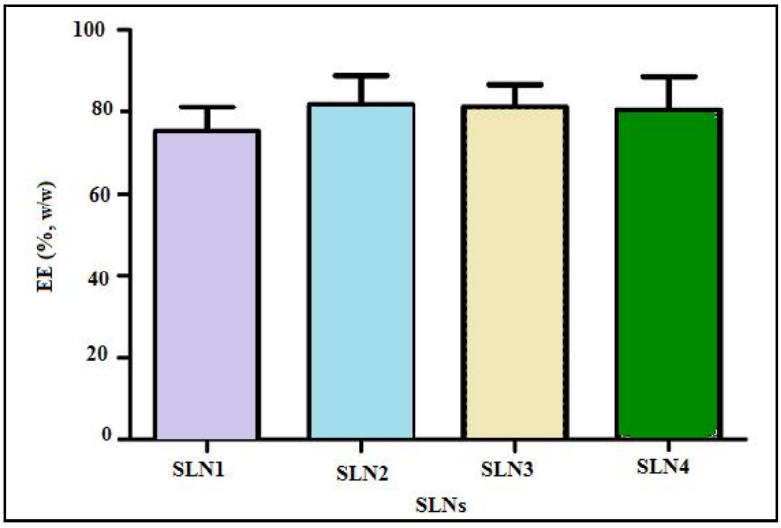

Figure 2: EE profile of SLNs loaded with quercetin.

3.2.1 Particle size analysis and zeta potential measurement

Particle size analysis and zeta potential measurement was determined through the standard protocol, successfully. The outcome of the study showed that the average particle size and zeta potential of the SLN2 were found as ~19.01 mV which represents the stability of the prepared nanoparticles. In particle size analysis, SLN unveiled with the mean particle diameter by ~339.8 nm, unimodal size distribution, a polydispersity index (PDI) by 0.171, intercept value 0.88 and 81% peak intensity. The outcome of the study has been depicted in Figure 3. The PDI of the nanoparticle reflects the dissemination factor which represents the aggregation index of the developed nanoparticles when PDI value would be < 0.5 (Yusuf *et al*., 2012).

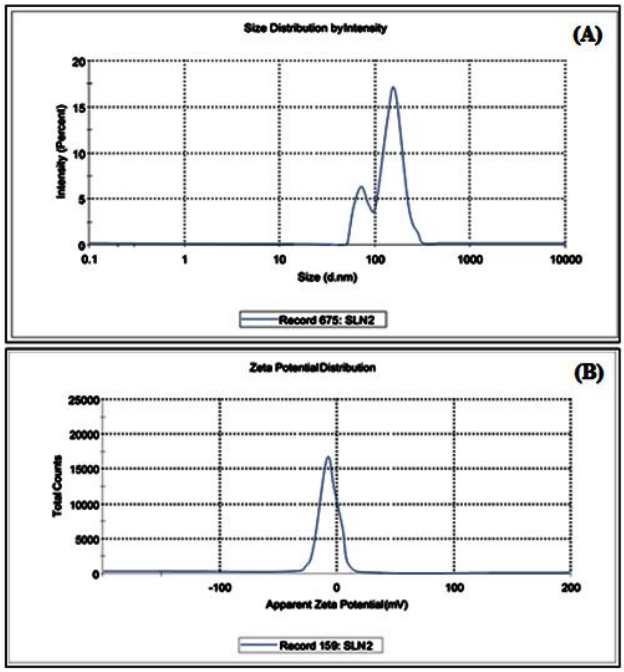

Figure 3: Particle size and zeta potential of SLN2.

3.2.2 FTIR of optimized SLN

FTIR analysis of the optimized SLNs (SLN2) was performed to determine the compatibility of the bases with the drug. The analysis was performed using the Perkin Elmer spectrophotometer. The results of the study showed several principal peaks at different frequency range at 3411.37 and 3291.01 cm^{-1}, represents the stretching of -OH and CH_2 group, two moderate peaks were appeared at the frequency range 1673.73 and 1618.23 cm^{-1}, represents the stretching vibration of -C=C and -C=O group while the peaks appeared at the frequency range 1207.99, 1172.69 and 827.83 cm^{-1} represents the stretching vibration of -C-O-C-, -C-H and aromatic hydrogen scissoring vibrations. The analysis showed least even no interaction of quercetin with the bases used for development of nanoparticles. The outcome of the study has been depicted in Figure 4. The results were matched with the previously reported literature which supports the present findings of the study (Kokalj Ladan *et al.*, 2017).

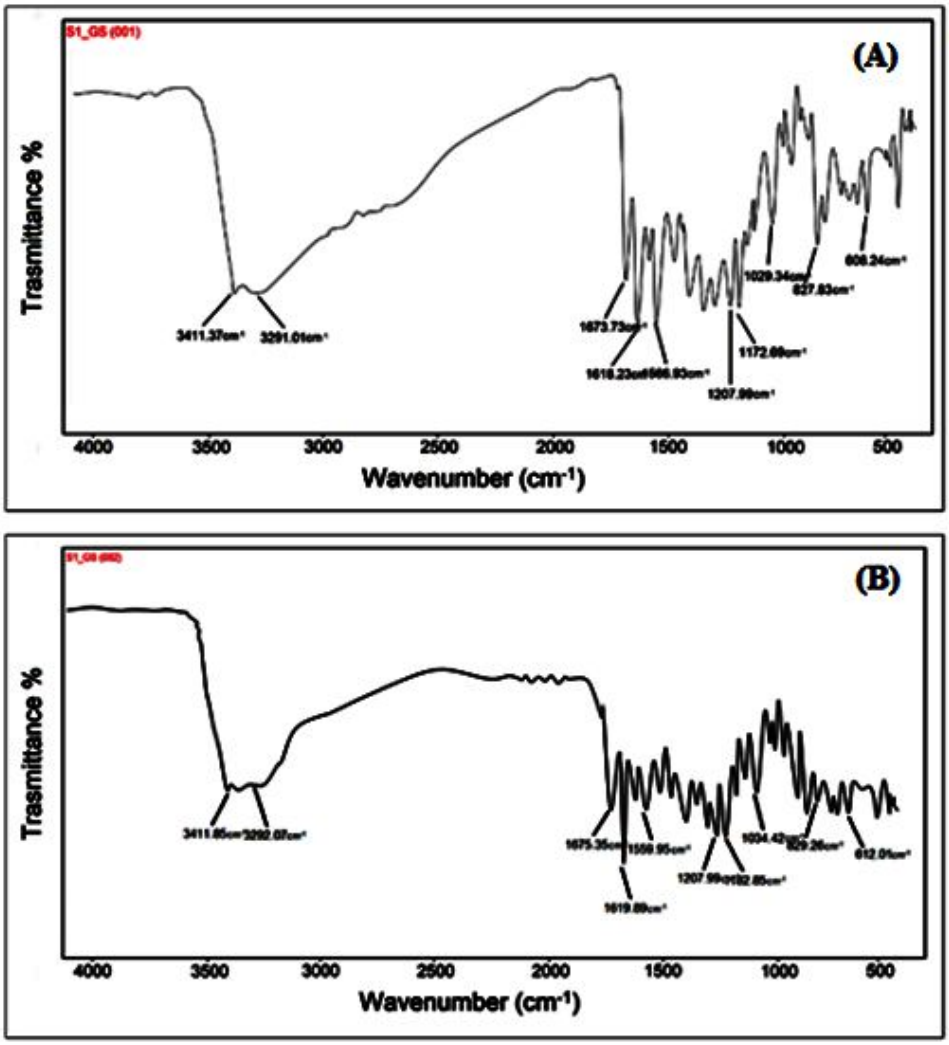

Figure 4: FTIR spectra of quercetin (A) and SLN2 (B).

3.3 Antidiabetic evaluation a-amylase and a-glucosidase inhibitory activity

a-amylase and a-glucosidase have been acknowledged as the characteristic enzymes which play an essential role in alleviation or elevation of diabetes. Most of the studies have been conducted based on the enzyme and substrate reaction. This is one of the most used and adaptable method to evaluate antidiabetic effect of the drug molecules (Capetti *et al.*, 2020; Gaurav *et al.*, 2020; Tahir *et al.*, 2016). In this present study, á-amylase and á-glucosidase inhibitory activity of the SLN2 were determined using the standard protocol. The analysis was conducted by an enzyme and substrate reaction method. During the study, a comparative analysis of SLN2 and quercetin was determined parallelly. The results showed that the maximum inhibition of the a-amylase by SLN2 and quercetin was found as 75.329 ± 6.238% and 59.384 ± 7.992%, respectively, while 58.9542 ± 5.7730% and 45.9380 ± 7.992% inhibition was found for a-glucosidase. The results represent that the developed SLN2 have significant even comparative higher inhibitory action than the control group of quercetin.The outcome of antidiabetic effect of quercetin has been summarized in Figure 5.

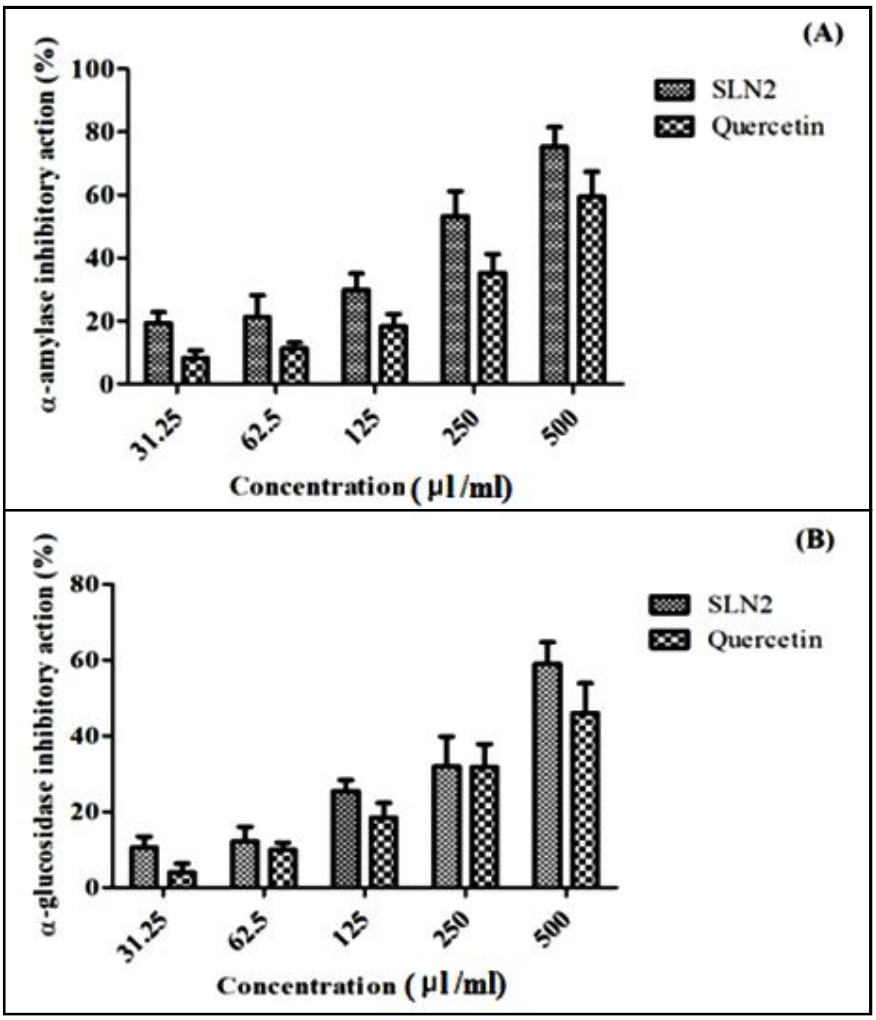

Figure 5: Antidiabetic evaluation of quercetin against a-amylase and a-glucosidase activity.

3.4 DPPH free radical scavenging activity

Oxidative stress has been characterized is the most deleterious condition by excessive production of the redox or free radicals. Although, our body system normally generates the free radicals due to normal cellular metabolism function. Excessive production of free radicals affects the function of vital organs, and thus reduction the activity of the body to function properly. Moreover, in a stressed condition due to excessive redox agents, the own body antioxidant defense system includes several enzymes such as catalase (CAT), superoxide dismutase (SOD) become compromised and unable to fight the excessive redox system. Natural products or their derived medicine act as the most prominent antioxidant agents. Thus, antioxidant effect of the developed SLN2 was determining using DPPH antioxidant activity. The outcome of the study showed that the scavenging effect of SLN2 and quercetin used as the control drug found comparable. The per cent scavenging effect of the developed SLN2 and quercetin was found as 63.329 ± 6.238% and 59.384 ± 7.992%, which showed no significant changes in per cent scavenging activity of both the sample. The graphical representation of the DPPH percentage scavenging activity of both the samples has been depicted in Figure 6.

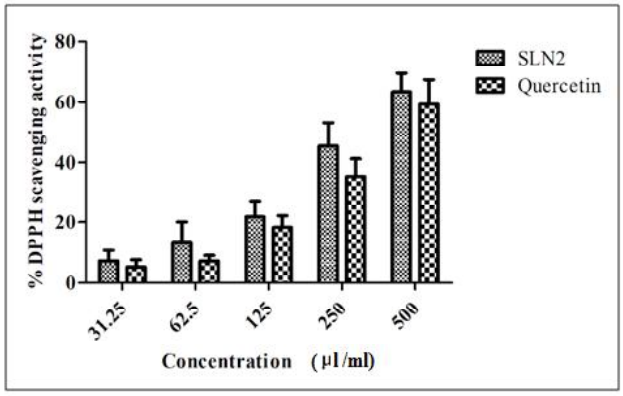

Figure 6: DPPH scavenging activity of quercetin.

3.5 Network pharmacology and gene ontology analysis

Network pharmacology is one of the most recent and exponentially used computational techniques for evaluation of biological interactivity of the drugs or compounds. It can be used for

multicomponent and multiproteins biochemical interaction. Based on the factors, quercetin was evaluated for its multimechanistic action for treatment of diabetes, even to evaluate the interaction with genes involved in pathophysiology of diabetes or associated disorders. Protein-protein interaction (PPI) and compound protein interaction (CPI) network was generated and the ligation efficacy of each gene was evaluated. In this analysis, a total of 56 genes were evaluated to determine their interaction of quercetin. The results showed that out of 56 genes, 31 genes were showing the interaction with quercetin, among them 17 genes were found least interacted while the rest genes were showing string PPI and CPI. In PPI network, number of nodes: 31, number of edges: 76, average node degree: 4.9, avg. local clustering coefficient: 0.649, expected number of edges: 29 and PPI enrichment *p*-value: 4.78e-13. The PPI network showed that each gene was partially interacted together, which represents their role in pathophysiology of diabetes and associated disorders. CPI network showed significant interaction of quercetin with several genomes such as IRS1, NOXs, TLRs, ILs, CASPs, PONs, UGTs, *etc.*, which plays an essential role in pathophysiology of diabetes and its associated disorders, even diabetes induced oxidative and inflammatory stress. It has been reported that quercetin play an essential role as anti-inflammatory and antioxidant agent. The diagrammatic representation of network pharmacology and gene ontology has been described in Figure 7.

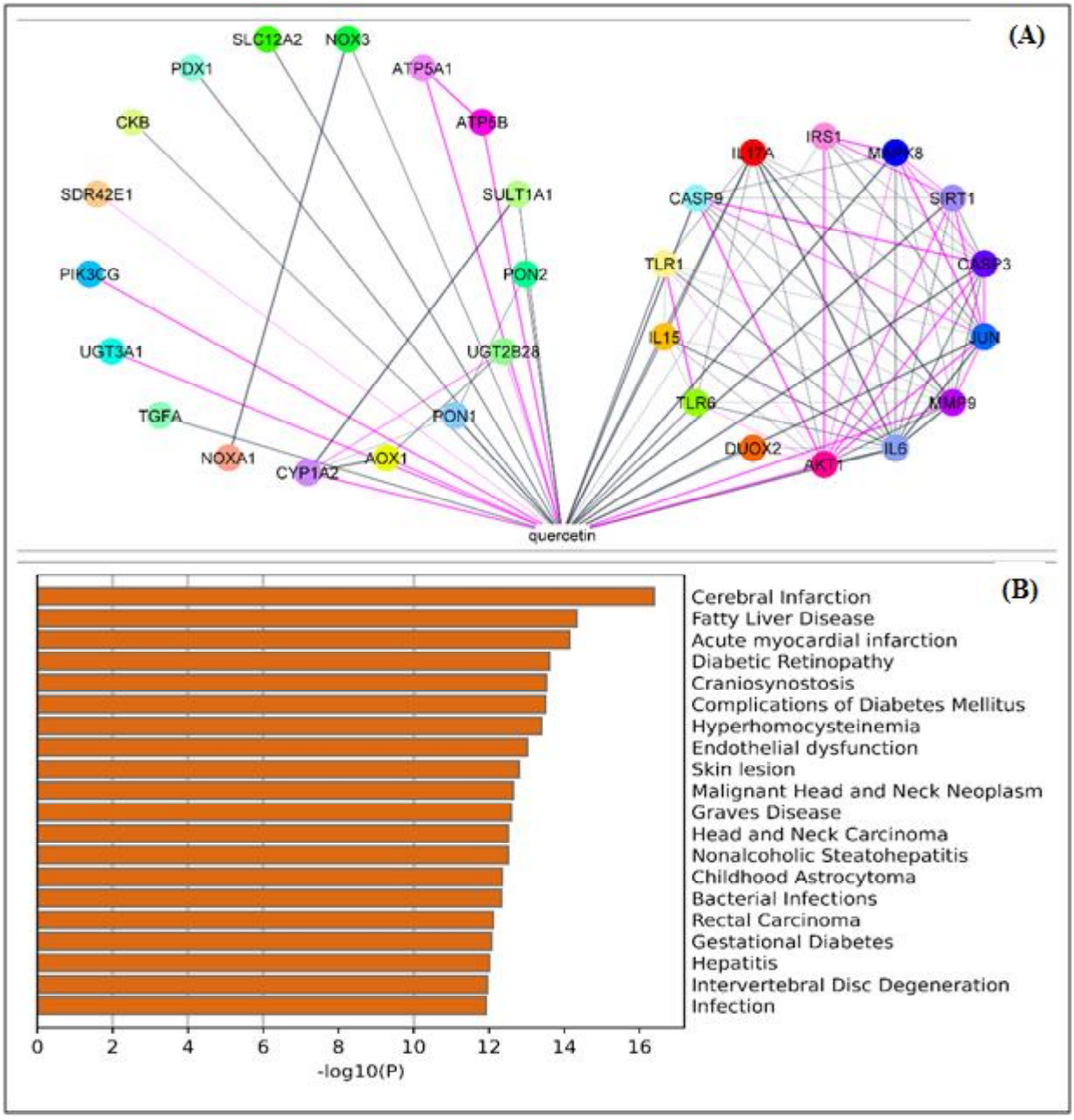

Figure 7: Network pharmacology (A) and gene ontology analysis of quercetin (B).

4. Discussion

Development of the promising therapeutic pharmaceuticals in critically needed to obviate the criticism of the lack of affordability, accessibility and compatibility among the present modern pharmaceuticals. The utilization of nanodrug delivery system-based pharmaceuticals is increasing exponentially in developing and developed countries (Mukherjee *et al*., 2009). Considering the serious incidence and prevalence of diabetes throughout the globe, the present aim of the study is associated with the investigation and development of a nanosystem-based formulation for alleviating diabetes. In this study, quercetin was selected as the active pharmaceutical ingredient as it has been reported as the most promising antidiabetic and antioxidant agent (Tang *et al*., 2017). In this study, preformulation studies were conducted to determine the originality, compatibility and method authenticity. The outcome of the lipophilicity and FTIR analysis were matched with the previously reported literatures which support the present findings of the study (Kokalj Ladan *et al*., 2017).

Postformulation studies were conducted successfully, entrapment efficiency (EE), particle size and zeta potential and compatibility analysis through FTIR were conducted to optimize the developed formulations. In EE analysis, it was found that among the developed formulations, SLN2 was found as the optimized SLN as it possessed the higher EE of the drug. Particle size and zeta potential analysis was assessed for the optimized SLNs (SLN2) for its better compatibility and accessibility. The analysis was performed at room temperature and favorable conditions using a Nano-ZS90 Zeta-sizer system, so that no further physicochemical changes could not occur. The stability of the nanoparticle is proportioned to the high zeta potential value as it could exerts a repulsive force between the nanoparticles (Tang *et al*., 2017). Phosphate buffer as the media for SLNs was used to evade compatibility issues or chemical changes. In antidiabetic activity, a-amylase and glucosidase inhibitory activity was determined in comparison with the control drug. The outcome of the study showed that SLN2 exhibited a comparatively higher inhibitory effect than the effect of the control drug. Previously reported studies showed that quercetin has a potential antidiabetic effect *via* regulation of enzymatic activity (Parveen *et al*., 2019; Song *et al*., 2020). In a study reported by Meng and his team evaluated the inhibitory effect of quercetin against a-amylase and a-glucosidase. The results showed that quercetin significantly inhibits the effect of a-amylase and a-glucosidase and reported the 770 mg ml^{-1} IC_{50} of quercetin (Meng *et al*., 2016).

Oxidative stress has been acknowledged major causing factor for any acute and chronic pathogenesis due to excessive production of the free radicals inside the body. Natural polyphenols are well known for their multi therapeutic action with prominent antioxidant activity (Gaurav *et al*., 2020). Most of the studies has been conducted to determine the antioxidant activity of pharmaceutical even phytopharmaceuticals. It has been reported that DPPH is one of the most used and abundant methods which not only used to determine the antioxidant activity of a single drug or herbal drug but even exponentially used in food industries to determine the antioxidant potential of food materials (Parsons, 2017). DPPH bioautography based analysis of pharmaceuticals especially the products derived from natural or herbal medicines is prominently used for direct screening of the active antioxidants compounds (Gaurav *et al*., 2020).

Network pharmacology analysis was conducted to explore the multimechanistic action of quercetin through direct and indirect interaction with the genes involved in the pathophysiology of diabetes and oxidative stress. The results showed that quercetin act as the most biologically active constituent which does not act as an antidiabetic, but also acts as an antioxidant, antiinflammatory and hepatoprotective agent. Several studies have been published that revealed that quercetin decreases cell migration and invasion by conquering the protein levels of MMP-2, p-Akt1, and MMP-9 thus constraining metastasis properties (Lu *et al*., 2018). PPARs are acknowledged as the potential gene which plays an important role in diabetic nephropathy and associated complications. The ligands for PPARs; namely, hypolipidemic PPAR alpha activators as well as antidiabetic thiazolidinedione PPAR gamma agonists, not only alleviate the renal disease but also offer the miscellaneous aspects of the treatment of disease associated with metabolic syndrome (Ruan *et al*., 2008). The endogenous antioxidant protein such as sestrin-2 (SESN2) strongly devoted in treatment of oxidative stress induce inflammation as well. It is reported that reduced expression of SESN2 in hyperglycemia exerts a protective effect on the podocyte and evade the cells from injury due to mitochondrial dysfunction, thus SESN2 can be the therapeutic target for the treatment of hyperglycemia-induced kidney disease (Lin *et al*., 2020). PON2 and PON3 belongs to the PONs family of proteins that significantly contribute to their role in dyslipidemia induced CKD. Quercetin significantly interacts with PONs proteins, and thus can be the targeted therapeutic regimen that may provide effective therapy in dyslipidemia induced CKD (Solati and Mahboobi, 2012). Form gene ontology (GO) analysis, it has been investigated that the screened genes are involved not only in the pathophysiology of diabetes, but also play an important role in cerebral infarction, fatty liver disease, diabetic retinopathy, complications of diabetes mellitus, endothelial dysfunction, *etc*., *via* regulation various oxidative, inflammatory and another cellular signaling pathway. Thus, it can be suggested that the developed quercetin SLNs play a significant role in alleviating hyperglycemia as well as the associated complication which directly and indirectly induces hyperglycemia.

5. Conclusion

The present study concludes that the developed and optimized SLNs formulation (SLN2) can be a promising pharmaceutical for alleviating diabetes and its associated complications. Although, further *in vivo* and clinical experiments are required to enhance the credibility of the developed nanopharmaceutical.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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