FORMULATION AND EVALUATION OF β-SITOSTEROL LOADED NIOSOMES FOR ENHANCED ANTIDIABETIC ACTIVITY: A POTENTIAL ALTERNATIVE IN TYPE 2 DIABETES MANAGEMENT

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ABSTRACT

Niosomes, vesicles formed by non-ionic surfactants, offer advantages in drug delivery by enhancing solubilization and encapsulating both lipophilic and hydrophilic drugs. This study focuses on β-sitosterol-loaded niosomes designed to protect its anti-diabetic properties. Formulated with Span™ 60, Span™ 80 and cholesterol, the niosomes showed high entrapment efficiency (90%) and favorable release kinetics. Stability studies confirmed their robustness in various conditions. *In vivo* experiments demonstrated significant improvements in body weight and blood glucose levels in diabetic rats. The optimized formulation (NF8) exhibited controlled release and substantial antidiabetic activity, suggesting its potential as an effective treatment for type 2 diabetes mellitus.

Keywords: Niosomes, non-ionic surfactants, β-sitosterol, release kinetics, blood glucose level

INTRODUCTION

Niosomes, lipid-based structures incorporating non-ionic surfactants and cholesterol, present a potential resolution to drug delivery challenges. They significantly enhance solubilization rates in gastrointestinal fluids, providing an effective platform for encapsulating drugs with both lipophilic and hydrophilic properties. Extensive research suggests that niosomes, mirroring the behavior of liposomes, extend drug circulation, modify distribution, and enhance metabolic stability. Their heightened affinity for phospholipids contributes to improved pharmacokinetic and pharmacodynamic properties, resulting in enhanced assimilation and prolonged effectiveness. Importantly, niosomes offer advantages, such as increased stability, improved penetration and cost reduction, compared to previous vesicular delivery systems. Given their exceptional permeability and superior drug encapsulation capacity, niosomes are the preferred choice for delivering substances like β-sitosterol. In this study, we developed niosomes incorporating β-sitosterol, with the objective of preserving its anti-diabetic activities.

The study is specifically focused on establishing a robust wall system for encapsulating β-sitosterol as the core within niosomes. This wall system employs the non-ionic surfactant Span™ 60 and cholesterol, acting as a stabilizer for the membrane and creating a bilayer barrier that enhances solute retention. Cholesterol plays a crucial role in reinforcing the adhesive interaction between the wall and core components. The study meticulously evaluates the performance of these niosome particles, examining entrapment efficiency, release profile and stability. Subsequently, the efficacy of β-sitosterol-loaded niosomes in treating diabetes is assessed through *in vivo* trials.

This novel approach, utilizing niosomes for the delivery of β-sitosterol, holds significant promise in enhancing anti-diabetic effects while effectively addressing challenges in drug delivery systems. The study aspires to contribute valuable insights into the development of robust and stable formulations for improved diabetes treatment[1].

MATERIALS AND METHODS

Materials

The materials listed below were obtained from the indicated sources without undergoing additional

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Table I: Formulation of various batches of niosomes

Formulation	Surfactant	Surfactant: Cholesterol	β-sitosterol	Surfactant	Cholesterol
NF1	Span™ 60	2:2	15 mg	200 mg	200 mg
NF2		2:3	15 mg	200 mg	300 mg
NF3		3:1	15 mg	300 mg	100 mg
NF4		3:2	15 mg	300 mg	200 mg
NF5		3:3	15 mg	300 mg	300 mg
NF6	Span™ 80	2:2	15 mg	200 mg	200mg
NF7		2:3	15 mg	200 mg	300 mg
NF8		2:4	15 mg	300 mg	400 mg
NF9		3:1	15 mg	300 mg	100 mg
NF10		3:2	15 mg	300 mg	200 mg
NF11		3:3	15 mg	200 mg	300 mg

purification. β-sitosterol was acquired from Yarrow Chem Products, Ghatkopar, Mumbai, Maharashtra, India. The remaining chemicals used were of analytical reagent grade.

Methods

PREPARATION OF β-SITOSTEROL FORMULATION

Niosomes were generated through the solvent injection method, utilizing ether as the solvent and incorporating β-sitosterol. This process involved employing nonionic surfactants (Span™ 60, Span™ 80) and varying concentrations of cholesterol, employing the ether injection technique. A solution was created by dissolving cholesterol and a surfactant in a mixture of 6 mL diethyl ether and 2 mL methanol, containing a measured quantity of β-sitosterol. The resulting solution was introduced into a 15 mL phosphate buffer solution with hydration using a micro-syringe, consistently delivering at a rate of 1 mL per minute while maintaining the buffer's pH at 7.4. The solution underwent continuous agitation using a magnetic stirrer, with the temperature consistently maintained between 60 and 65°C. Details of the formulae and their compositions are presented in Table I[2].

CHARACTERIZATION

The analysis encompassed the examination of pure β-sitosterol, followed by an evaluation of β-sitosterol niosomes, focusing on parameters such as particle size, entrapment efficiency and *in vitro* drug release. Additionally, *in vivo* investigation on diabetic rats was conducted to assess the biological activity of the substance.

CHARACTERIZATION OF β-SITOSTEROL LOADED NIOSOMES

PARTICLE SIZE AND SHAPE

The surface morphology and structure of niosomes was scrutinized using scanning electron microscopy (SEM). The experiments used a Nova Nanosem 450 SEM model. Subsequently, the formulation containing β-sitosterol-loaded niosomes was diluted with phosphate buffer for further analysis.

% ENTRAPMENT EFFICIENCY (EE)

The entrapment efficiency, or the proportion of content, was calculated by subtracting the amount of drug not entrapped or free in the supernatant from the initial amount of the drug. Then, this value was divided by the total amount of medication integrated into the nano-carrier formulation[3].

Method

- Taken 1 mL of sample (noisome).
- The item was stored in the refrigerator at temperature of -20 °C for a duration of 24 h.
- Subsequently, allowed to defrost at ambient temperature.
- To extract the unbound drug from the solution, centrifugation was carried out at a speed of 14000 revolutions per minute for 40 minutes at a temperature of 5 °C.
- The specimen was washed with phosphate-buffered saline (PBS) at a pH of 7.4, and then the resulting aqueous fraction was collected.

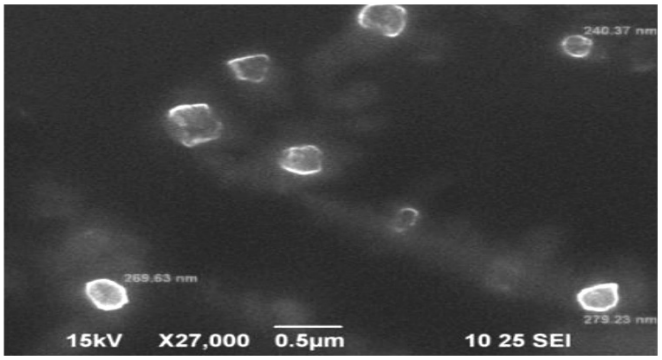

Fig. 1: SEM images of Niosome (NF8)

The combined amount of β-sitosterol present in the niosomes and the amount of β-sitosterol trapped within the niosomes were both measured and analysed using a UV spectrophotometric technique. The calculation of encapsulation efficiency was performed using the following equation.

$$\% \text{ Entrapment Efficacy} = \frac{\text{Total amount of drug substituted by unentrapped drug}}{\text{Total amount of drug}} \times 100$$

IN VITRO DRUG RELEASE PROFILE

The liberation of β-sitosterol from niosomes using dialysis equipment was assessed. The setup was finalized by connecting a 15 kD hydrated dialysis membrane to the funnel of the diffusion cell assembly, forming the experimental configuration of a dialysis bag and a receptor. We encapsulated 3 mL of niosomes within a dialysis bag and tightly secured them with a dialysis clip. Then, the bag was inserted into a beaker, serving as the receiving compartment, and filled it with 250 mL of phosphate-buffered saline (PBS) at a pH of 7.4. The PBS solution was vigorously stirred at a rate of 50 revolutions per minute (37±1 °C) using a magnetic stirrer. The sampling process involved periodically extracting 4 mL of the sample for 72 h and then replacing it with an equivalent volume of fresh PBS. The gathered samples underwent analysis for drug content using a UV spectrophotometer at a peak wavelength of 245 nm. The investigation used PBS as a control solution[4,5].

STABILITY STUDIES

For three months, β-sitosterol-loaded niosomes were kept in containers with tight lids and were subjected to certain storage conditions to test their stability. The storage required refrigeration at a temperature of 4°C±2°C and ambient temperature conditions of 25 ±2°C, with a relative humidity (RH) of 60±5%. This storage period lasted for three months. A thorough inspection was conducted at the start and end of the three-month storage period. These evaluations surprisingly revealed no noticeable alterations, providing strong proof that the composition remained stable throughout the entire storage duration. This highlights the formulation's ability to withstand changes in the environment and demonstrates its potential for long-term use without altering its structure or function.

ANTI-DIABETIC ACTIVITY OF β-SITOSTEROL LOADED NIOSOMES

ANIMALS

We acquired adult male Sprague-Dawley (SD) rats weighing between 160 and 200 g from CSIR-CDRI in Lucknow. The Institutional Animal Ethics Committee (IAEC) of Integral University Lucknow granted clearance to the protocol, with the assigned approval number IU/IAEC/20/24.

EXPERIMENTAL PROTOCOL

The experimental animals were categorised into five groups, each containing six participants. The rats were given a diet consisting of 84.3% standard laboratory chow, 5% lard, 10% yolk powder, 0.2% cholesterol, and 0.5% bile salt for two weeks. Diabetes was induced in all groups, except the control group (group 1), using a standardised approach.

INDUCTION OF TYPE-2 DIABETES

A fresh solution of streptozotocin (STZ) was made by dissolving it in a sodium citrate buffer at a concentration of 0.1 M. Rats in experimental groups II–V were administered a solitary intraperitoneal (i.p.) injection of this solution. The individuals' fasting blood glucose (FBG) levels were recorded before administering the intraperitoneal STZ dosage. The fasting blood glucose (FBG) levels were reassessed after 72 h of STZ treatment and animals were selected for the remaining experimental procedures if their blood glucose level (BGL) was 250 mg dL^{-1} or higher[6].

Initial evaluations of body weight and blood glucose levels were conducted for all animal groups, and the findings were documented. The treatment protocol, outlined in Table II, specifies the interventions for each group. Group I was subjected to a 1% carboxymethyl cellulose (CMC) treatment. Rats with diabetes in Groups II, III, IV, and V were given different substances orally for 15 days. Group II received 1% CMC at a dose of 10 mg kg^{-1}, Group III received metformin at a dose of 500 mg kg^{-1}, Group IV received pure β-sitosterol dissolved in 0.5 mL

of olive oil at a dose of 15 mg kg^{-1}, and Group V received β-sitosterol-loaded niosomes at a dose of 15 mg kg^{-1}.

Table II: Treatment schedule

Group	Treatment	Dose/route/duration
Group I	1% CMC (Carboxymethyl cellulose)	10 mL kg^{-1}, p.o. (per oral), once a day for 15 days
Group II	Diabetic rat + 1% CMC	10 mL kg^{-1}, p.o. once a day for 15 days
Group III	Diabetic rat + metformin	500 mg kg^{-1}, p.o., once a day for 15 days
Group IV	Diabetic rat + β-sitosterol	15 mg kg^{-1}, p.o., once a day for 15 days
Group V	Diabetic rat + β-sitosterol niosome formulation (NF8)	15 mg kg^{-1}, p.o., once a day for 15 days

The experiment concluded by recording the ultimate body weight and blood glucose levels of all the animal groups.

PARAMETERS ASSESSED

Body weight

The initial and ultimate body weights of the rats in each group were documented [7].

Blood glucose level

Blood samples were acquired from the rats' tail vein, and the blood glucose concentration was assessed using a glucometer with a diagnostic kit.

STATISTICAL ANALYSIS

The results were reported as the average ± standard error of the mean (SEM), derived from a sample size of six. The statistical analysis was performed using GraphPad Prism (Version 9.00 for Windows 7) and a two-way analysis of variance (ANOVA) was applied. The p-values below 0.05 ($p<0.001$) were considered significant.

RESULTS

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF NIOSOMES

The formulation and characterization of niosomes loaded with β-sitosterol were carried out.

Physical characterization (Particle size)

The morphology of niosomes underwent analysis and confirmation through scanning electron microscopy (SEM), aligning with the hydrodynamic particle size. The particles displayed a spherical morphology, visually resembling one another, as illustrated in Fig. 1.

% Entrapment efficiency (EE)

The % entrapment efficiency of β-sitosterol liposomes was observed between 44±1.24 and 90±1.15, as shown in Table III and Fig. 2.

Table III: Percentage entrapment efficiency of various batches of β-sitosterol niosomes

Sr. No.	Formulation code	% Entrapment efficiency
1	NF1	44±1.24
2	NF2	50±0.95
3	NF3	62±1.46
4	NF4	71±2.43
5	NF5	76±2.12
6	NF6	67±1.96
7	NF7	58±1.05
8	NF8	90±1.15
9	NF9	49±1.38
10	NF10	57±1.49
11	NF11	46±2.05

All the results are reported as the average of three determinations ± standard deviation (SD).

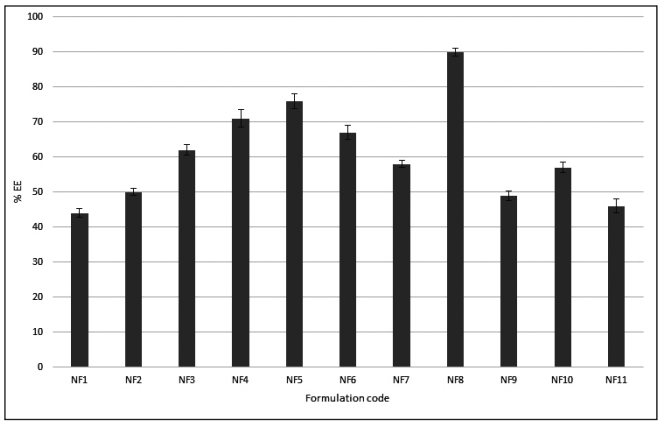

Fig. 2: Percentage entrapment efficiency of various batches of β-sitosterol niosome

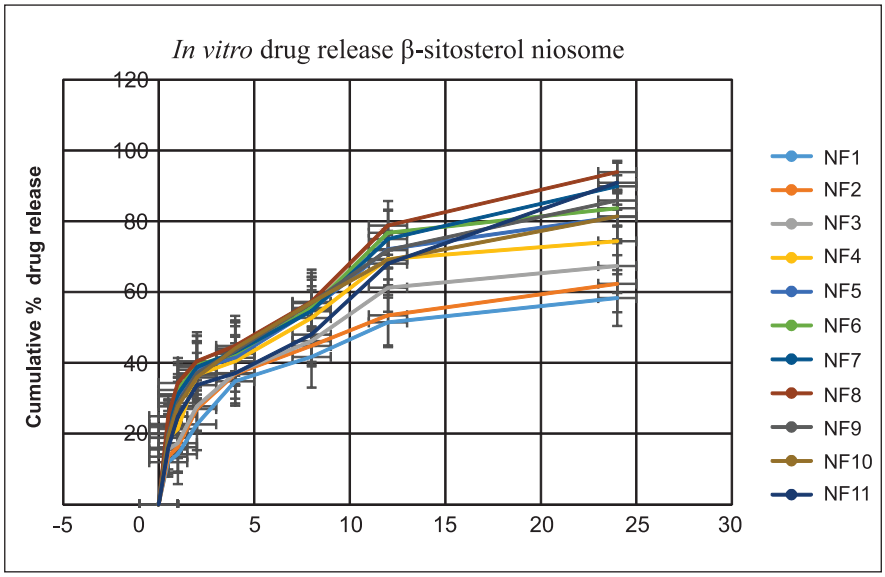

Fig. 3: *In vitro* drug release of various batches of β-sitosterol niosome

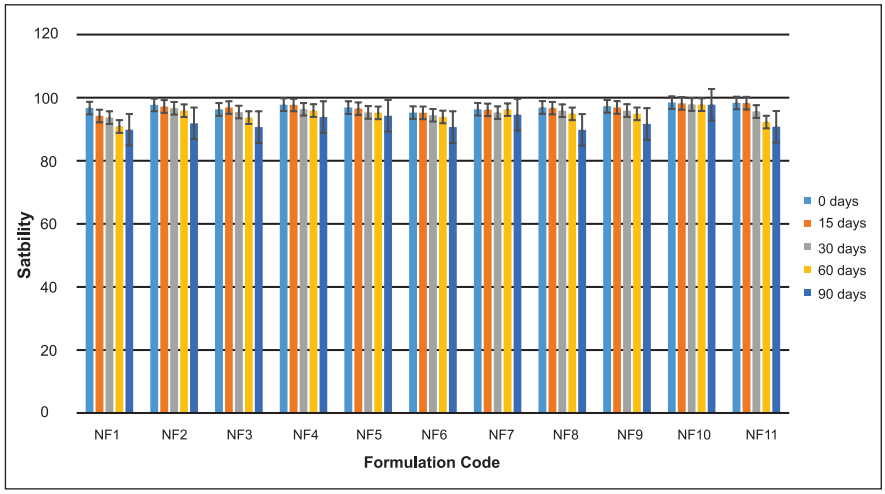

Fig. 4: Stability of niosomes on different days

IN VITRO DRUG RELEASE

An evaluation was performed to analyze the drug release kinetics of β-sitosterol-loaded niosomes. Linear regression analysis of β-sitosterol release data indicated a diffusion-regulated mechanism. The initial phase exhibited a rapid release, likely attributed to membrane-bound β-sitosterol, followed by sustained release from the encapsulated form. The drug release was monitored for 24 h in aqueous solutions, and a significant cumulative drug release (CDR) of 93.89±5.97% was achieved in phosphate buffer with a pH of 7.4, as illustrated in Fig. 3. Mathematical models, including zero-order kinetics, first-order kinetics, Higuchi kinetics, and Korsemeyer-Peppas, elucidated and confirmed the release mechanism of β-sitosterol from niosomes. The formulation exhibited a continuous and extended-release pattern, emphasizing its potential for maintaining an optimal concentration for *in vivo* effectiveness, as detailed in Table IV. The observed first-order release kinetics further support the sustained release behavior of the present β-sitosterol composition. This composition holds promise for ensuring prolonged therapeutic efficacy.

Table IV: Curve fitting data of release rate profile of niosomes formulation

Formulation	Zero order (R2)	First order (R2)	Higuchi (R2)	Korsemeyer - Peppas (R2)
NF 8	0.8193	0.9803	0.9608	0.3596

STABILITY STUDIES

The NF8 formulation was introduced into a container and subjected to controlled storage conditions, including 4°±2°C/60±5% RH and room temperature (25°±2°C/60±5% RH), over a three-month period. Following this duration, the samples underwent examination to assess physical characteristics, drug

Table V: Effect of β-sitosterol on body weight

Group	"0" days	"14" days
Group I	185.17 ± 4.47	206.54 ± 5.32
Group II	188.76 ± 4.27	148.72 ± 6.70
Group III	181.42 ± 5.47	201.62± 7.40
Group IV	182.43 ± 6.60	190.61 ± 5.47
Group V	183.71 ± 6.61	193.41 ± 4.60

Values are expressed as mean ±SEM (n=6).

Table VI: Assessment of anti-diabetic activity

Group name	Treatment	Plasma glucose (mg dL^{-1})	
		Before treatment	After treatment
Group I	Control	91.3 ± 7.12	90.0 ± 5.06
Group II	Diabetes control	250.1 ± 13.61	263.3 ± 12.07
Group III	Diabetic + metformin (500 mg kg^{-1} body weight)	259.01 ± 13.65	90.8 ± 7.38
Group IV	Diabetic + β-sitosterol (15 mg kg^{-1} body weight pure drug)	260.05 ± 18.12	110.8 ± 8.09
Group V	Diabetic + β-sitosterol niosome (15 mg kg^{-1} body weight)	265.2 ± 15.67	100.5 ±7.21

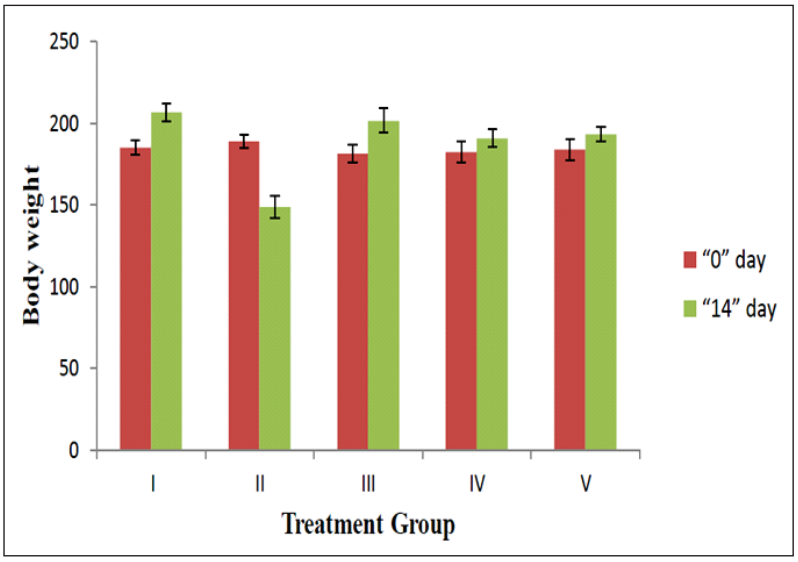

Fig. 5: Effect of β-sitosterol on body weight

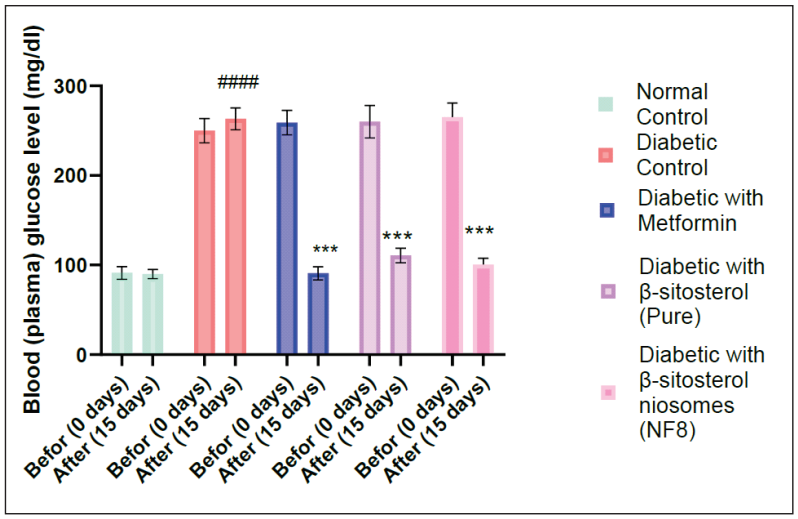

Fig. 6: Effect of β-sitosterol on blood glucose level

containment and *in vitro* release behavior. The entrap-ment efficiency was found to be 90±1.05%, with a release percentage of 93.89±4.97%. Notably, there were no significant alterations observed in the formulation's stability over the three-month period, as illustrated in Fig. 4.

ANTI-DIABETIC ACTIVITY OF β-SITOSTEROL LOADED NIOSOMES

Effect of β-sitosterol on body weight

Effect of β-sitosterol on body weight in diabetic rats, Group I exhibit moderate variability in weight, characterized by an initial increase followed by mixed results on Day 14. Group II, on the other hand, demonstrates a consistent trend of weight reduction, indicating the presence of potential interventions or conditions that lead to weight loss. In comparison between Groups III and IV, Group III showing a slight increasing trend. Lastly, Group V displays an overall pattern of slightly weight gain, particularly noticeable between Day 0 and Day 14, shows in Table V and Fig. 5.

Effect of β-sitosterol on blood glucose levels

The impact of β-sitosterol on blood glucose levels was evaluated in diabetic rats. Diabetic control rats exhibited a notable elevation in blood glucose levels in comparison to normal control rats receiving normal saline. In Group IV, the administration of pure β-sitosterol at a

dosage of 15 mg kg^{-1}, and in Group V, the administration of the niosomes formulation (NF8) at the same dose, led to a significant and dose-dependent reduction in blood glucose levels ($p<0.001$). However, rats treated solely with β-sitosterol did not display a statistically significant change in blood glucose levels compared to the control group, as detailed in Table VI and Fig. 6.

DISCUSSION

This study examines the effects of therapeutic interventions on plasma glucose levels in rats with experimentally produced diabetes by the use of streptozotocin (STZ). STZ is frequently employed in diabetes research because of its specific targeting of β-cells, resulting in reduced insulin levels and hyperglycemia, which replicates the pathology of diabetes. STZ hampers the functioning of β-cells and is linked to elevated levels of NO and peroxynitrite, which worsen its toxicity. The experimental groups, as indicated in Table V, exhibit diverse patterns in weight fluctuations during a 14-day duration.

Group I had moderate weight variability, Group II revealed steady weight loss, Group III exhibited a slight upward trend, and Group V showed a consistent moderate weight gain. The topic centres around the impact of therapeutic interventions on plasma glucose levels.

Group I exhibited a marginal elevation in plasma glucose levels within a span of 15 days, possibly attributed to natural fluctuations or unmanageable variables. The Control Group (II) demonstrated a substantial increase in glucose levels, confirming the progressive nature of diabetes when not properly controlled. Group III, which received metformin treatment, exhibits a significant decrease in plasma glucose levels. Group IV, when administered with β-sitosterol, likewise exhibited a notable reduction. Group V, which received β-sitosterol niosomes (NF8), exhibited the most significant decrease in glucose levels, suggesting that this treatment may be more effective than previous therapies.

CONCLUSION

The objective of this study was to create and evaluate niosomes that contain β-sitosterol to maintain their anti-diabetic characteristics. Significantly, the niosomes, specifically NF8, demonstrated favorable attributes such as a high level of entrapment efficiency and sustained release of medication. This can be due to the use of Span™ 60 and cholesterol. The formulation's robustness was confirmed through three-month stability testing done in different settings. Experiments conducted on live diabetic rats showed that β-sitosterol niosomes, particularly NF8, resulted in a notable gain in body weight and a decrease in blood glucose levels when compared to the control group. The findings indicate that β-sitosterol niosomes have the potential to be a promising therapeutic strategy for diabetes. To summarise, this study effectively developed niosomes containing β-sitosterol, which demonstrated improved stability and potential effectiveness in the treatment of diabetes. Additional research and clinical trials are necessary to examine their practical use in managing diabetes.

REFERENCES

1. Sharma P. K. Saxena P. Jaswanth A. Chalamaiah M. and Balasubramaniam A.: Anti-diabetic activity of lycopene niosomes: Experimental observation, **J. Pharm. Drug Dev.,** 2017, 4, 103.
2. Sailaja A. K. and Shreya M.: Preparation and characterization of naproxen loaded niosomes by ether injection method., **Nano Biomed. Eng.,** 2018, 10, 174–180.
3. Mokhtar M. Sammour O. A. Hammad M. A. and Megrab N. A.: Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes, **Int. J. Pharm.,** 2008, 361, 104–111.
4. Zange R. Li Y. and Kissel T.: Biocompatibility testing of ABA triblock copolymers consisting of poly (L-lactic-co-glycolic acid) A blocks attached to a central poly (ethylene oxide) B block under *in vitro* conditions using different L929 mouse fibroblasts cell culture models, **J. Control. Release**, 1998, 56, 249–258.
5. Kushwaha A. S. Repka M. A. and Narasimha Murthy S.: A novel apremilast nail lacquer formulation for the treatment of nail psoriasis, **AAPS Pharm. Sci. Tech.,** 2017, 18, 2949–2956.
6. Zhou Z. Li L. Yang Y. Xu X. and Huang Y.: Tumor targeting by pH-sensitive, biodegradable, cross-linked N-(2-hydroxypropyl) methacrylamide copolymer micelles, **Biomaterials**, 2014, 35, 6622–6635.
7. Gandhi G. R. and Sasikumar P.: Antidiabetic effect of *Merremia emarginata* Burm. F. in streptozotocin induced diabetic rats, **Asian Pac. J. Trop. Biomed.,** 2012, 2, 281–286.