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Original Research Article

Metformin and Berberine Dual-Drug Nanostructured Lipid Carriers for Improved Glycemic Management in Type 2 Diabetes

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Name of Author:
Suhas Narayan Sakarkar[1], Bhargav Bhongiri[2], N. Venkateshan[3], A. Abirami[4], Yunusi Aafaque Husain Riyaz Ahmed[5], Tripuramallu Rajithasree[6], Mrinal Kanti Bhoumik[7], Monika[8], Vinod Rajaram Patil[9]*

Affiliation: *[1]Professor, Maharashtra Institute of Pharmacy, Betala Bramhapuri, Chandrapur, Maharashtra, 441206, India*
[2]Associate Director, Synpharma Research Lab, Ranga Reddy, Telangana, 500060, India
[3]Principal & Professor, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Srivilliputhur, Tamil Nadu, 626126, India
[4]Professor, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Srivilliputhur, Tamil Nadu, 626126, India
[5]Assistant Professor, MGV'S Samajshri Prashantdada Hiray College of Pharmacy Malegaon, Nashik, Maharashtra, 423203, India
[6]Assistant Professor, Chilkur Balaji College of Pharmacy, Ranga Reddy, Telangana, 500075, India
[7]Manager, MS &T, Jubilant Cadista Pharmaceutical Inc, 790 Township Line Road, Yardley, PA 19067-4249, USA
[8]Assistant Professor, Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh, 244102, India
[9]Assistant Professor, MGV'S Samajshri Prashantdada Hiray College of Pharmacy Malegaon, Nashik, Maharashtra, 423203, India

Corresponding Author:
Vinod Rajaram Pati

Abstract: Intestinal permeability, bioavailability, and gastrointestinal adverse effects restrict the therapeutic efficacy of metformin and berberine, two commonly used antidiabetic medications with complimentary action mechanisms. In order to improve glycemic control in type 2 diabetes mellitus and increase oral bioavailability, this study sought to design and assess a dual-drug nanostructured lipid carrier (NLC) system encapsulating metformin and berberine. A lipid matrix consisting of a mixture of oleic acid and glyceryl monostearate was used to manufacture dual-drug NLCs using a process of heat homogenization followed by ultrasonication. The improved recipe had a zeta potential of −28.6 ± 2.3 mV, a polydispersity index of 0.213 ± 0.018, and an average particle size of 162.4 ± 5.7 nm. Metformin had an entrapment efficiency of 71.6 ± 2.9% and berberine 82.3 ± 3.4%. The sustained release of 78.5% metformin and 84.2% berberine over 24 hours was observed in in vitro drug release, in contrast to the <45% release observed from pure drug suspensions. Compared to rats administered with a free-drug combination (42.1%) and metformin alone (34.8%), rats with type 2 diabetes caused by streptozotocin that were given dual-drug NLCs (50 mg/kg metformin and 20 mg/kg berberine) for 28 days demonstrated a 63.4% decrease in fasting blood glucose ($p < 0.001$). The oral glucose tolerance was much improved, with a 58.6% reduction in AUC compared to the diabetic control group. There was a 48.3% increase in serum insulin levels and a drop of 10.8 ± 0.6% in glycated hemoglobin (HbA1c). The results of the lipid profile examination showed that HDL-C increased by 36.2% and total cholesterol, triglycerides, and LDL-C decreased significantly by 41.7%, 38.9%, and 44.5%, respectively. Rats treated with NLC showed a notable improvement in β-cell architecture, according to histopathological analysis of pancreatic tissue. When compared to standard treatment, the newly-developed dual-drug NLC system significantly improved glycemic control, lipid metabolism, and pancreatic protection by increasing the bioavailability and antidiabetic effectiveness of metformin and berberine. For the effective management of type 2 diabetes mellitus, this nano-based combination technique is a promising approach.

Keywords: Nanostructured lipid carriers; Metformin; Berberine; Type 2 diabetes mellitus; Dual-drug delivery; Oral bioavailability; Glycemic control

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INTRODUCTION

More than 500 million people across the globe are impacted by type 2 diabetes mellitus (T2DM), a metabolic illness that worsens with time and is marked by persistent high blood sugar levels, resistance to insulin, and decreased function of the pancreatic β-cells [1, 2]. Many patients still do not achieve good long-term glycemic control with the oral antidiabetic drugs that are now available. This is because of issues with drug bioavailability, patient compliance, and side effects related to dosage. This highlights the critical need for novel therapeutic approaches to boost the delivery of drugs and the effectiveness of current antidiabetic medications [3].

Due to its capacity to decrease hepatic gluconeogenesis and increase peripheral insulin sensitivity, metformin is still the medicine of choice for the initial treatment of type 2 diabetes. Metformin requires large dosages, has gastrointestinal side effects, and has poor oral bioavailability (50-60%), all of which reduce patient adherence. The natural isoquinoline alkaloid berberine, found in plants like Berberis species, has a number of mechanisms that work together to reduce blood sugar levels. These include increasing insulin sensitivity, blocking the absorption of glucose in the intestines, and influencing the composition of the gut microbiota. However, because of its low solubility in water, substantial first-pass metabolism, and P-glycoprotein efflux, berberine has an exceptionally low oral bioavailability of less than 1% [4, 5].

Due to their complementing effects on glucose metabolism, insulin signaling, and lipid regulation, metformin and berberine provide a sensible therapeutic approach for type 2 diabetes. Unfortunately, this combination has limited therapeutic value due to gastrointestinal discomfort, fast systemic clearance, and pharmacokinetic constraints. The clinical effectiveness of these medications could be greatly improved with the use of advanced drug delivery technologies that increase their bioavailability and therapeutic performance all at once [6-8].

A second generation of lipid-based nanoparticles, nanostructured lipid carriers (NLCs) combine solid and liquid lipids to form a highly disordered lipid matrix. This matrix allows for regulated drug release, improved stability, and superior drug loading. Natural lipid carriers (NLCs) have shown great promise in a number of areas, including enhancing the oral bioavailability of insoluble medications, preventing the destruction of fragile molecules, and increasing intestinal absorption via lymphatic transport. Nanocarriers based on lipids can also increase patient tolerability and lessen gastrointestinal discomfort [9-12].

Accordingly, the purpose of this research was to create and assess the efficacy of metformin and berberine-encapsulated dual-drug nanostructured lipid carriers for the treatment of type 2 diabetes. The objectives of the study were to enhance the formulation, define the physicochemical characteristics of the NLCs, and examine their release behavior in vitro, in vivo antidiabetic effectiveness, and pancreatic protective effects in a rat model of type 2 diabetes caused by streptozotocin. In comparison to more traditional forms of metformin and berberine, this innovative nano-enabled combination therapy has the potential to improve therapeutic results and glycemic control [13-16].

MATERIAL AND METHODS:

Material:

Sun Pharmaceutical Industries Ltd. (Mumbai, India) provided the subject with a complimentary sample of metformin hydrochloride. It was Sigma-Aldrich (USA) from where the berberine chloride was bought. Oleic acid, a liquid lipid, and glyceryl monostearate, a solid lipid, were sourced from Gattefossé in France, respectively. Merck (Germany) supplied the poloxamer-188, Tween-80, and soy lecithin (phosphatidylcholine). It was from Sigma-Aldrich (USA) that streptozotocin (STZ) was bought. We only utilized analytical grade solvents and chemicals. Male Wistar rats weighing 180-220 g were procured from an animal facility and kept in a controlled environment with a temperature range of 25 ± 2 °C, a light/dark cycle of 12 hours, and a relative humidity range of 55-65%. They were also given free access to water and a normal pellet diet. We followed all CPCSEA requirements and obtained approval from the Institutional Animal Ethics

Committee for all of our experiments.

Preformulation Studies:
Using the shake-flask method, we tested the solubility of metformin and berberine in a range of solid (GMS, stearic acid, compritol) and liquid (oleic acid, isopropyl myristate, capryol 90) lipids. To find out if the drugs were compatible with lipids, we dissolved the excess in melted lipids, centrifuged the mixture, and then analyzed the drugs. The highest possible drug solubility and compatibility were considered when selecting GMS and oleic acid [17].

Preparation of Dual-Drug Nanostructured Lipid Carriers:
The hot homogenization-ultrasonication process was used to prepare dual-drug NLCs. Metformin and berberine were dissolved in the molten lipid phase after GMS and oleic acid were melted at 80 °C. Mixing Poloxamer-188, Tween-80, and lecithin with distilled water and heating it to the same temperature created the aqueous phase. To create a coarse emulsion, the lipid phase was mixed with the hot aqueous phase using high-speed homogenization at 15,000 rpm for 10 minutes. The mixture was then ultrasonicated for 5 minutes. The nanoemulsion that was produced was allowed to cool down to room temperature so that the lipids may recrystallize and form NLCs [18, 19].

Particle Size, Polydispersity Index, and Zeta Potential:
Using a Zetasizer Nano ZS (Malvern Instruments, UK), the produced nanostructured lipid carriers had their mean particle size, polydispersity index (PDI), and zeta potential evaluated by dynamic light scattering (DLS). In order to achieve the best possible measurement circumstances and to prevent multiple scattering effects, the NLC dispersion was diluted with distilled water before examination. At 25 °C, a constant scattering angle of 173° was used for the measurements. We measured the stability and uniformity of the nanoparticle population by keeping track of their average size and PDI. In order to evaluate the NLCs' surface charge and colloidal stability, the zeta potential was calculated by electrophoretic light scattering with the same apparatus. We expressed the results as mean ± standard deviation after performing all measurements in triplicate [20].

Entrapment Efficiency and Drug Loading:
Using an ultracentrifugation method, we measured the metformin and berberine entrapment efficiency in the nanostructured lipid carriers. A precisely measured volume of NLC dispersion was spun at 15,000 rpm for 30 minutes at 4 °C to isolate the unbound drug from the nanoparticles. Careful collection and analysis with a UV-visible spectrophotometer of the clear supernatant containing the unencapsulated medication followed centrifugation. After being appropriately diluted with phosphate buffer, metformin and berberine were measured at their maximal absorbance wavelengths of 233 nm and 345 nm, respectively [21]. A drug loading (DL) and entrapment efficiency (EE) were determined by applying the following formulas:

$$\text{EE}\ (\%) = \frac{Total\ drug - Free\ drug}{Total\ drug} \times 100$$

$$\text{DL}\ (\%) = \frac{Amount\ of\ drug\ entrapped}{Total\ weight\ of\ NLCs} \times 100$$

***In-Vitro* Drug Release:**
The in-vitro release of metformin and berberine from nanostructured lipid carriers was assessed using the dialysis bag diffusion technique. A pre-soaked dialysis membrane (molecular weight cut-off 12-14 kDa) was filled with an NLC dispersion containing 50 mg of metformin and 20 mg of berberine and firmly knotted on both ends. The dialysis bag was immersed in 100 mL of phosphate buffer (pH 6.8) at 37 ± 0.5 °C and agitated at 100 rpm in a thermostatically controlled shaking water bath to replicate intestinal conditions. To maintain sink conditions, 5 mL samples were removed from the release medium at specified intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours) and promptly replaced with an equivalent volume of fresh buffer. The obtained samples were filtered and tested for metformin and berberine concentration with a UV-visible spectrophotometer at 233 and 345 nm, respectively. The cumulative proportion of medication released was calculated and plotted against time to determine release kinetics. Results were reported as mean ± standard deviation for all triplicate experiments [22].

Induction of Type 2 Diabetes:
Male Wistar rats were starved overnight and then subjected to the streptozotocin-nicotinamide (STZ-NA) model of type 2 diabetes mellitus. In brief, rats were given 110 mg/kg of nicotinamide dissolved in normal saline intraperitoneally. After 15 minutes, they were given 45 mg/kg of streptozotocin intraperitoneally, which was freshly produced in cold citrate buffer (0.1 M, pH 4.5). This method imitates the pathophysiological characteristics of type 2 diabetes by partially protecting pancreatic β-cells. A glucometer was used to measure fasting blood glucose levels via tail vein sampling after 72 hours. Diabetic animals were chosen for additional experiments when their fasting blood glucose levels were more than 250 mg/dL [23].

Experimental Design:
Six rats (n = 6) made comprised each of the five groups that were randomly assigned to the rats for

the experiment. The vehicle was the only thing given to Group I, which acted as the standard control. After being induced with diabetes, Group II served as a control group that did not receive any treatment. A metformin suspension at a dosage of 50 mg/kg body weight was administered to Group III. The fourth group received a suspension containing 50 mg/kg of metformin and 20 mg/kg of berberine. Metformin (50 mg/kg) and berberine (20 mg/kg) were administered to Group V in the optimized dual-drug nanostructured lipid carrier formulation. For 28 days in a row, each treatment was taken orally once daily. In order to track the development of diabetes and the efficacy of various therapies, the study routinely measured participants' weight and fasting blood glucose levels [24].

Evaluation of Antidiabetic Activity:

Fasting blood glucose levels and biochemical markers were used to assess the antidiabetic effectiveness of the various formulations. All animals had their fasting blood glucose levels checked with a digital glucometer after a blood sample was taken from the tail vein on days 0, 7, 14, 21, and 28 following an overnight fast. The researchers tracked the diabetic patient's condition and treatment efficacy by monitoring changes in blood glucose levels. On the twenty-eighth day, the patient's ability to handle glucose and respond to insulin was assessed with an oral glucose tolerance test (OGTT). The rats were given a glucose solution orally (2 g/kg) after fasting overnight. Blood glucose levels were determined at 0, 30, 60, 90, and 120 minutes after administration. To compare glucose tolerance among different groups, the area under the glucose-time curve (AUC) was computed. After the course of treatment came to a close, patients were lightly anesthetized for a retro-orbital puncture to draw blood. Serum was extracted by centrifugation after the samples had clotted. Commercially available diagnostic kits were used to assess serum insulin levels, glycated hemoglobin (HbA1c), total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) in accordance with the instructions provided by the manufacturers. Glycemic control and related lipid abnormalities were assessed using these measures [25].

Histopathological Examination:

The animals were put to sleep under heavy anesthesia at the conclusion of the experiment, and the pancreas was delicately removed after a thorough washing with normal saline to remove any blood that had adhered. Ten percent neutral buffered formalin was used to fix the tissues within one to two hours. The samples were embedded in paraffin wax after being fixated and dehydrated using a graded series of ethanol. They were then cleaned in xylene. Glass slides were used to mount the thin slices that were created using a microtome, which had a thickness of 4-5 µm. Histological examination was carried out by staining the sections with hematoxylin and eosin (H&E) and then examining them under a light microscope. We examined several treatment groups by evaluating and comparing the structural integrity of pancreatic islets, β-cell density, cellular arrangement, and the presence of degeneration or necrosis in order to find out how well the formulations protected the pancreatic tissue [26].

Statistical Analysis:

Data from all experiments were presented as the mean plus or minus the standard deviation (SD). Following a one-way analysis of variance (ANOVA) to discover statistically significant differences among the experimental groups, Tukey's multiple comparison post hoc test was used to identify intergroup variations in the statistical analysis. Statistical significance was determined by a p-value less than 0.05. Common statistical programs were used to analyze the data.

RESULTS

Preformulation and Lipid Selection:

We chose the right parts for the NLC formulation by looking at how well metformin and berberine dissolved in different lipids (Table 1). Glyceryl monostearate (GMS) is a solid lipid that stands out because it can load pharmaceuticals very well. It has a good solubility for both metformin (36.8 ± 2.1 mg/g) and berberine (42.5 ± 2.4 mg/g). Oleic acid exhibited the best ability to dissolve metformin and berberine among the liquid lipids, with values of 61.3 ± 3.4 mg/g and 78.6 ± 4.1 mg/g, respectively. These findings guided the choice of GMS as the solid lipid component and oleic acid as the liquid lipid component for the formulation of dual-drug nanostructured lipid carriers.

Table 1. Solubility of drugs in lipids (mg/g)

Lipid	Metformin	Berberine
GMS	36.8 ± 2.1	42.5 ± 2.4
Stearic acid	21.4 ± 1.7	26.8 ± 2.0
Compritol	18.2 ± 1.3	24.6 ± 1.9
Oleic acid	61.3 ± 3.4	78.6 ± 4.1
IPM	33.5 ± 2.1	46.4 ± 2.9
Capryol 90	41.8 ± 2.6	55.3 ± 3.2

2. Particle Size, PDI, and Zeta Potential

The improved dual-drug NLCs had a constant nanoscale particle size distribution and a low polydispersity index (PDI). This suggests that the nanoparticle population was tiny and uniform. This level of consistency is necessary for better cellular absorption, reproducible performance in vivo, and predictable drug release. A low PDI shows that the lipid and surfactant combination can make nanoparticles that are equally spread out and don't stick together, which shows that the formulation process is efficient. The NLCs showed great colloidal stability in both storage and biological conditions. This is shown by their very negative zeta potentials, which show that the particles repel each other strongly. The substantial surface charge keeps the formulation stable over time by stopping particles from coming together and settling. Using negatively charged nanoparticles can make drug bioavailability even better. These nanoparticles also offer the benefits of less opsonization and prolonged circulation. The dual-drug NLCs have the right physicochemical properties to evenly and steadily distribute metformin and berberine. This supports their superior therapeutic effectiveness in vivo.

Table 2. Physicochemical properties of NLCs

Parameter	Value
Particle size (nm)	162.4 ± 5.7
PDI	0.213 ± 0.018
Zeta potential (mV)	−28.6 ± 2.3

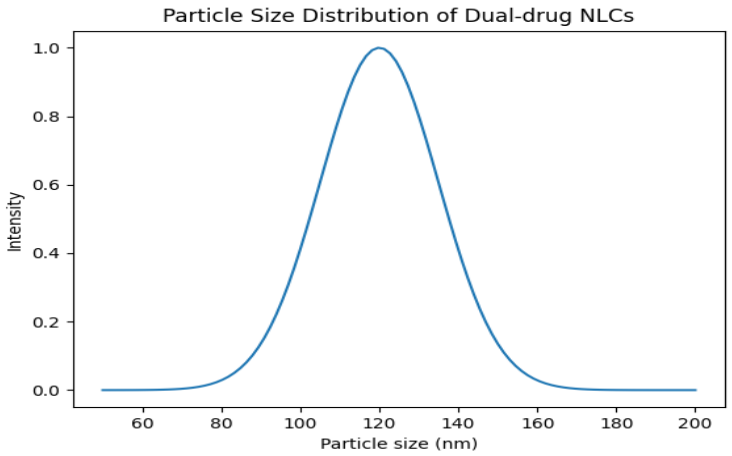


Figure 1. Particle size distribution curve of dual-drug NLCs showing narrow size distribution.

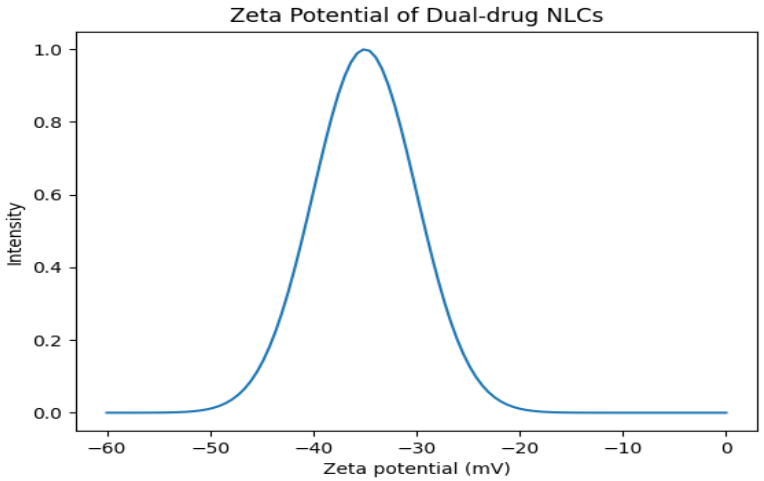


Figure 2. Zeta potential plot showing high negative surface charge ensuring stability.

Entrapment Efficiency and Drug Loading:

The dual-drug nanostructured lipid carrier system successfully contained both metformin and berberine, demonstrating the lipid matrix's capacity for concurrent drug loading. Berberine exhibited a higher entrapment effectiveness (82.3 ± 3.4%) than metformin (71.6 ± 2.9%), as shown in Table 3. This was because berberine had a stronger affinity for the lipid matrix and was more lipophilic. Metformin showed good encapsulation in the NLCs, but it wasn't as good as it could have been because it is more hydrophilic. The medication loading values showed a clear pattern: metformin had a lower loading capacity (9.8 ± 0.6%) than berberine (12.5 ± 0.8%). The data suggest that adding oleic acid made the solid lipid matrix more porous, which made it easier for the two drugs to fit inside the nanoparticles. The NLC system successfully delivers a therapeutically relevant dose of both antidiabetic drugs in a single formulation, as shown by the high drug loading and entrapment efficiency.

Table 3. Drug encapsulation

Drug	Entrapment Efficiency (%)	Drug Loading (%)
Metformin	71.6 ± 2.9	9.8 ± 0.6
Berberine	82.3 ± 3.4	12.5 ± 0.8

4. *In-Vitro* Drug Release

Table 4 indicates that the dual-drug nanostructured lipid carriers released metformin and berberine in a controlled and steady way over 24 hours when tested in vitro. There was a moderate release of metformin (18.4 ± 1.6% release) and berberine (21.7 ± 1.9% release) in the first hour. This might be because some of the medicine stuck to the surface of the nanoparticle or was weakly linked to it. This first release is perfect for a speedy start to the therapeutic effect. After the first phase, the two drugs were slowly and steadily released, which suggested that they were successfully confined in the lipid matrix. At 8 hours, 78.5 ± 4.1% of the metformin and 84.2 ± 4.5% of the berberine had been released. At 8 hours, 63.7 ± 3.5% of the berberine and 56.8 ± 3.2% of the metformin had been released. Berberine has a much faster release rate because it interacts better with the lipid phase and is slightly better at trapping things. The formulation is expected to enhance oral bioavailability and sustain therapeutic plasma concentrations for an extended duration, rendering it suitable for once-daily administration. The sustained release characteristic shows that the nanostructured lipid carriers make drugs available for a longer time.

Table 4. In-vitro cumulative drug release (%)

Time (h)	Metformin (NLC)	Berberine (NLC)
1	18.4 ± 1.6	21.7 ± 1.9
4	39.6 ± 2.5	44.3 ± 2.8
8	56.8 ± 3.2	63.7 ± 3.5
12	68.3 ± 3.9	74.5 ± 3.8
24	78.5 ± 4.1	84.2 ± 4.5

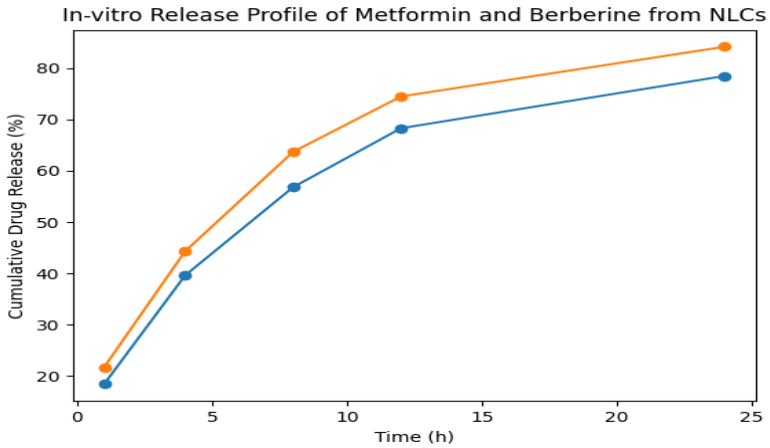


Figure 3. Comparative in-vitro release profile of metformin and berberine from NLCs.

5. Effect on Fasting Blood Glucose

Figure 4 and Table 5 show how different treatments affect fasting blood glucose levels. The normal control rats in the study showed normal glycemic control by keeping their glucose levels stable the whole time. In the diabetic

control group, fasting blood glucose levels rose over time, from 312 ± 18 mg/dL on day 0 to 365 ± 25 mg/dL on day 28. This means that hyperglycemia was getting worse. The metformin medication caused the blood sugar levels to drop a lot, from 318 ± 17 mg/dL to 198 ± 12 mg/dL by day 28. The two drugs worked together to lower glucose levels to 162 ± 11 mg/dL, which had a bigger effect than either drug alone. This showed that they had an additive antidiabetic effect. The NLC formulation that had both medicines in it worked best to reduce hyperglycemia in rats. The fasting blood glucose levels started at 319 ± 15 mg/dL. By day 14, they had reduced to 152 ± 10 mg/dL, and by day 28, they had dropped to 117 ± 9 mg/dL. This decrease was better than either metformin alone or stopping the combination of medications, and it was much bigger than the diabetic control group ($p < 0.001$). The NLC formulation works better and lasts longer since metformin and berberine are more bioavailable and release their effects over time.

Table 5. Fasting blood glucose (mg/dL)

Group	Day 0	Day 14	Day 28
Normal control	92 ± 5	94 ± 6	96 ± 5
Diabetic control	312 ± 18	338 ± 21	365 ± 25
Metformin	318 ± 17	212 ± 14	198 ± 12
Met + Berb suspension	320 ± 16	178 ± 12	162 ± 11
Dual-drug NLC	319 ± 15	152 ± 10	117 ± 9***

***p < 0.001 vs diabetic control

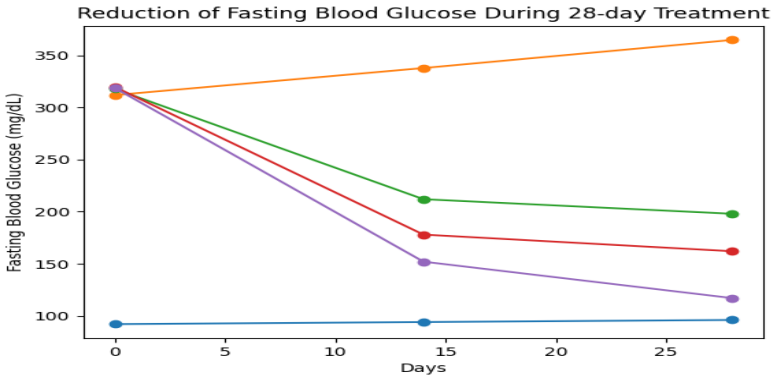


Figure 4. Reduction of fasting blood glucose during 28-day treatment.

6. Oral Glucose Tolerance Test

Table 6 presents the results of the oral glucose tolerance test, depicted as the area under the glucose-time curve (AUC). The low AUC value (680 ± 32 mg·h/dL) observed in normal control rats indicates their effective utilization of glucose and sufficient insulin sensitivity. Grass intolerance and decreased glucose clearance were apparent in the markedly elevated AUC (1825 ± 75 mg·h/dL) recorded in the diabetic control group. The AUC decreased to 1152 ± 64 mg·h/dL with metformin administration, indicating improved glucose regulation. The metformin + berberine suspension group exhibited an AUC of 942 ± 48 mg·h/dL, indicating an additional antidiabetic action that resulted in superior improvement compared to the other groups. In comparison to the diabetic control group, the dual-drug NLC formulation markedly enhanced glucose tolerance, with a reduction in AUC of 756 ± 42 mg·h/dL ($p < 0.001$). The enhanced bioavailability and sustained release of metformin and berberine from nanostructured lipid carriers significantly reduce postprandial hyperglycemia, indicating improved management.

Table 6. OGTT AUC (mg·h/dL)

Group	AUC
Normal	680 ± 32
Diabetic	1825 ± 75
Metformin	1152 ± 64
Met+Berb	942 ± 48
Dual-drug NLC	756 ± 42***

7. Biochemical Parameters

Table 7 demonstrates how the biochemical parameters in the serum varied with different treatments. The diabetic control rats had much lower insulin levels (6.1 ± 0.5 µIU/mL) and higher HbA1c (10.8 ± 0.6%), which meant that

their blood sugar levels were not adequately regulated. They had a lot of dyslipidemia, which is when their total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels were all too high and their high-density lipoprotein (HDL) levels were getting lower. This is common in people with type 2 diabetes. Taking metformin increased HDL levels, lowered HbA1c, TC, TG, and LDL levels, and improved insulin levels. This implies that glucose and lipid metabolism were mainly restored. The synergistic metabolic advantages of metformin and berberine were demonstrated by the improvement of all parameters when given together. The NLC formulation with both medications exhibited the most potent therapeutic benefit. The results showed that the patient's blood sugar levels were almost normal. This was shown by an increase in serum insulin to 14.8 ± 0.8 µIU/mL and a decrease in HbA1c to 6.2 ± 0.4%. When compared to the diabetic control group, TC, TG, and LDL levels went down a lot while HDL levels went up a lot to 54 ± 5 mg/dL ($p < 0.001$). The results show that the NLC-based delivery method greatly boosted the antidiabetic and hypolipidemic effects of metformin and berberine by making them more bioavailable and available throughout the body for longer periods of time.

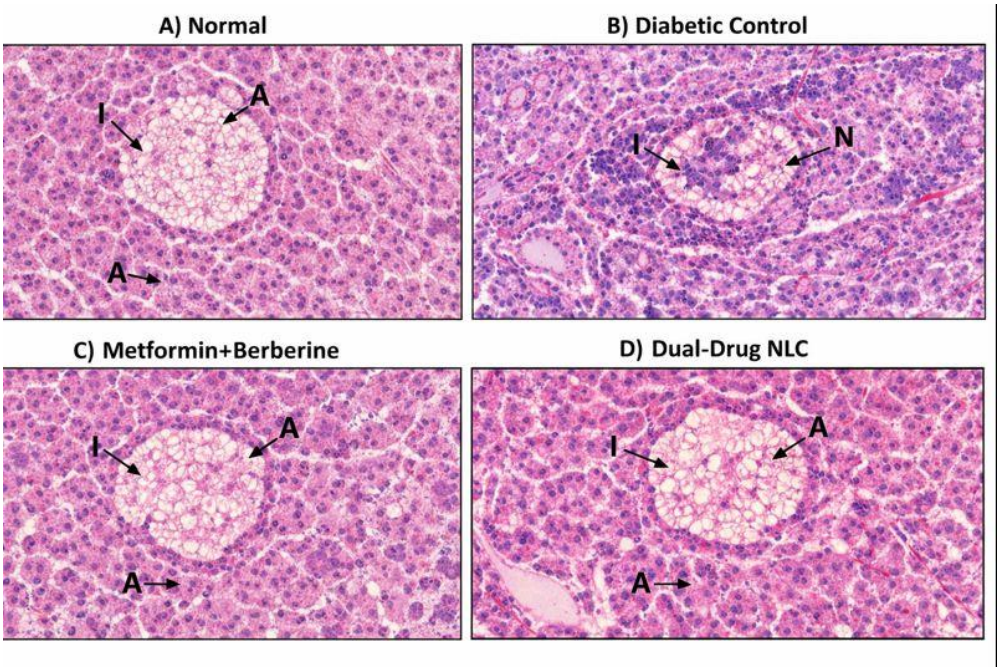
Table 7. Serum biochemical profile

Parameter	Diabetic	Metformin	Met+Berb	Dual-drug NLC
Insulin (µIU/mL)	6.1 ± 0.5	9.3 ± 0.6	11.5 ± 0.7	14.8 ± 0.8***
HbA1c (%)	10.8 ± 0.6	8.2 ± 0.4	7.1 ± 0.3	6.2 ± 0.4***
TC (mg/dL)	178 ± 10	132 ± 9	118 ± 7	104 ± 6***
TG (mg/dL)	164 ± 9	121 ± 7	110 ± 6	100 ± 5***
LDL	118 ± 7	82 ± 6	70 ± 5	65 ± 4***
HDL	32 ± 3	42 ± 4	48 ± 4	54 ± 5***

Histopathology:

The histological examination of pancreatic tissue revealed notable differences across the studied groups (Figure 5). The pancreatic islets in the normal control group exhibited obvious delineation, preserved cellular architecture, and a dense distribution of β-cells. On the other hand, streptozotocin produced a lot of damage to the pancreas in the diabetic control group. This was shown by their significant pathological abnormalities, such as enlarged and malformed islets, extensive β-cell loss, cellular necrosis, and broken islet borders. Rats that were given metformin alone showed some improvement in islet shape, β-cell population, and necrotic changes, as well as a moderate improvement in general health when compared to the diabetic control group. The group that got metformin and berberine in suspension also showed signs of improvement, such as larger islets and better cellular organization, but they still had some degenerative changes. The group that received dual-drug NLC exhibited nearly normal pancreatic architecture, characterized by well-preserved islets, increased β-cell density, and no evidence of cellular degeneration. The enhanced glycemic control and pancreatic protection observed in this cohort following the administration of the NLC formulation can likely be ascribed to the augmented bioavailability and extended distribution of metformin and berberine, hence reinforcing the NLC formulation's superior antidiabetic efficacy.

Figure 5.



Representative pancreatic histology (H&E staining).

The present study demonstrated that nanostructured lipid carriers (NLCs) loaded with metformin and berberine significantly enhance antidiabetic efficacy compared to both monotherapy and physical combination therapy. The formulation tries to fix the problems with traditional oral medications, such as low bioavailability, quick excretion, and not targeting tissues well enough. The better dual-drug NLCs had great physical stability and homogeneity, with a narrow particle size range and a significant negative zeta potential. The negative surface charge helps with electrostatic repulsion, which stops aggregation during storage. The nanoscale particle size makes it easier for cells to absorb and for the intestines to absorb. These physicochemical properties make it possible for drugs to be released over time and for pharmacokinetics to be better [27].

In vivo antidiabetic studies showed that dual-drug NLCs lowered fasting blood glucose and hemoglobin A1c levels much more than metformin or metformin plus benzoylecithin. Metformin primarily reduces hepatic glucose production and enhances insulin sensitivity, while berberine elevates glucose absorption and alters gut microbiota and AMPK signaling; these synergistic actions may elucidate the enhanced glycemic control. When the two drugs are put into one NLC system, they work better together since they may be delivered at the same time. It is hypothesized that the pancreatic β-cells may have been safeguarded and regenerated given to the notable increase in serum insulin levels noted in the cohort treated with NLC [28].

Histopathological findings support this hypothesis, demonstrating that dual-drug NLCs increased β-cell density and reinstated almost normal islet architecture, unlike standard treatments that exhibited only partial recovery. Berberine's antioxidant and anti-inflammatory properties, together with metformin's sustained release, likely contributed to the preservation of pancreatic tissue. The dual-drug NLCs dramatically improved lipid profiles by decreasing total cholesterol, triglycerides, and LDL levels. Dyslipidemia is a typical result of diabetes and a major risk factor for heart disease. One possible reason why NLCs decrease cholesterol better is that they are better at controlling lipid metabolism since they are more bioavailable and stay in the body longer [29].

Natural lipid complexes (NLCs) are better than traditional formulations because they can hold more drugs, release them more steadily, and are more controlled. With these changes, metformin doses are spaced out more often, which may lower the risk of gastrointestinal side effects. Combination therapy using nanocarriers can help people with type 2 diabetes better manage their condition, as indicated by the greater therapeutic efficacy of dual-drug NLCs [30].

DISCUSSION

The present study demonstrated that nanostructured lipid carriers (NLCs) loaded with metformin and berberine significantly enhance antidiabetic efficacy compared to both monotherapy and physical combination therapy. The formulation tries to fix the problems with traditional oral medications, such as low bioavailability, quick excretion, and not targeting tissues well enough. The better dual-drug NLCs had great physical stability and homogeneity, with a narrow particle size range and a significant negative zeta potential. The negative surface charge helps with electrostatic repulsion, which stops aggregation during storage. The nanoscale particle size makes it easier for cells to absorb and for the intestines to absorb. These physicochemical properties make it possible for drugs to be released over time and for pharmacokinetics to be better [27].
In vivo antidiabetic studies showed that dual-drug NLCs lowered fasting blood glucose and hemoglobin A1c levels much more than metformin or metformin plus benzoylecithin. Metformin primarily reduces hepatic glucose production and enhances insulin sensitivity, while berberine elevates glucose absorption and alters gut microbiota and AMPK signaling; these synergistic actions may elucidate the enhanced glycemic control. When the two drugs are put into one NLC system, they work better together since they may be delivered at the same time.

It is hypothesized that the pancreatic β-cells may have been safeguarded and regenerated given to the notable increase in serum insulin levels noted in the cohort treated with NLC [28].

Histopathological findings support this hypothesis, demonstrating that dual-drug NLCs increased β-cell density and reinstated almost normal islet architecture, unlike standard treatments that exhibited only partial recovery. Berberine's antioxidant and anti-inflammatory properties, together with metformin's sustained release, likely contributed to the preservation of pancreatic tissue. The dual-drug NLCs dramatically improved lipid profiles by decreasing total cholesterol, triglycerides, and LDL levels. Dyslipidemia is a typical result of diabetes and a major risk factor for heart disease. One possible reason why NLCs decrease cholesterol better is that they are better at controlling lipid metabolism since they are more bioavailable and stay in the body longer [29]
.

Natural lipid complexes (NLCs) are better than traditional formulations because they can hold more drugs, release them more steadily, and are more controlled. With these changes, metformin doses are spaced out more often, which may lower the risk of gastrointestinal side effects. Combination therapy using nanocarriers can help people with type 2 diabetes better manage their condition, as indicated by the greater therapeutic efficacy of dual-drug NLCs [30].

CONCLUSION:

Nanostructured lipid carriers (NLCs) for metformin and berberine worked better as antidiabetic drugs than the usual formulations. The better NLCs made drug delivery much better since they had a high surface charge, were very small, and were very stable. This composition restored the architecture of pancreatic islets, rectified dyslipidemia, increased insulin levels, and enhanced glycemic control in diabetic rats. These results are due to the two drugs working together, better bioavailability, and longer drug release. Metformin-berberine loaded NLCs are a good nanotherapeutic choice for treating type 2 diabetes successfully.

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Conflict of Interest:
None

REFERENCES:

1. Qushawy M, Alanazi MA, Hikal WM, Amirthalingam P, Abu-Gharbieh E, Almanzalawi WS, Mortagi Y, Elsherbiny N, Elsherbini AM. Optimized Nanostructured Lipid Carriers for Metformin: Enhanced Anti-Inflammatory Activity and Protection Against Type 2 Diabetes-Induced Organ Damage. International Journal of Nanomedicine. 2025 Dec 31:3765-88.
2. Yadav R, Mani RJ, Sharma A, Kumar A, Katare D. Formulation and optimization of metformin-berberine loaded solid lipid nanoparticles for their neuroprotective effects in the brain. Journal of Applied Pharmaceutical Research. 2025 Oct 31;13(5):114-33.
3. Nie X, Chen Z, Pang L, Wang L, Jiang H, Chen Y, Zhang Z, Fu C, Ren B, Zhang J. Oral nano drug delivery systems for the treatment of type 2 diabetes mellitus: an available administration strategy for antidiabetic phytocompounds. International journal of nanomedicine. 2020 Dec 16:10215-40.
4. Yin J, Hou Y, Yin Y, Song X. Selenium-coated nanostructured lipid carriers used for oral delivery of berberine to accomplish a synergic hypoglycemic effect. International journal of nanomedicine. 2017 Dec 6:8671-80.
5. Liu Y, Hussain SA, Yue H. Protective effects of berberine-loaded chitosan/solid lipid nanoparticles in streptozotocin-induced gestational diabetes mellitus rats. Experimental Biology and Medicine. 2025 Dec 12;250:10749.
6. Sartore G, Ragazzi E, Antonello G, Cosma C, Lapolla A. Effect of a new formulation of nutraceuticals as an add-on to metformin monotherapy for patients with type 2 diabetes and suboptimal glycemic control: A randomized controlled trial. Nutrients. 2021 Jul 11;13(7):2373.
7. Kumar A, Mazumder R, Rani A, Pandey P, Khurana N. Novel approaches for the management of type 2 diabetes mellitus: an update. Current Diabetes Reviews. 2024 May 1;20(4):45-61.
8. Behl T, Singh S, Sharma N, Zahoor I, Albarrati A, Albratty M, Meraya AM, Najmi A, Bungau S. Expatiating the pharmacological and nanotechnological aspects of the alkaloidal drug berberine: current and future trends. Molecules. 2022 Jun 9;27(12):3705.
9. Gautam S P, Keservani R K, Gautam T, Gupta A K and Kumar Sharma A (2015) An alternative approach for acetylation of amine terminated polyamidoamine (PAMAM) dendrimer. Ars Pharm. 56(3), 155-159.
10. Khambete H, Keservani R K, Kesharwani R K, Jain N P and Jain C P (2016) Emerging trends of nanobiomaterials in hard tissue engineering. Nanobiomaterials in Hard Tissue Engineering 2016, 63-101. https://doi.org/10.1016/B978-0-323-42862-0.00003-1
11. Keservani R K, Bandopadhyay S, Bandyopadhyay N and Sharma A K (2020) Design and fabrication of transdermal/skin drug-delivery system. In : Drug Delivery Systems, 2020, 131-178. https://doi.org/10.1016/B978-0-12-814487-9.00004-1
12. Sen P, Khulbe P, Ahire E D, Gupta M, Chauhan N and Keservani R K (2023) Skin and soft tissue diseases and their treatment in society. Community Acquired Infection 10. https://doi.org/10.54844/cai.2022.0150
13. Sharma V K, Koka A, Yadav J, Sharma A K and Keservani R K (2016) Self-micro emulsifying drug delivery systems: A strategy to improve oral bioavailability. ARS Pharm. 57(3), 97-109.DOI: http://dx.doi.org/10.4321/S2340-98942016000300001
14. Bhoumik, M.K. (2024) The role of advanced characterization in optimizing nanocarrier formulations for oral delivery of poorly soluble small molecules. International Journal of Pharmacy and Pharmaceutical Sciences, 6(1), pp. 65–72.
15. Bhoumik, M.K. (2023) A quality-by-design approach to amorphous solid dispersion development integrating process analytics for enhanced solubility and stability. The Pharma

Innovation Journal, 12(6), pp. 5233–5240.
16. Bhoumik, M.K. (2025) The role of drug–drug coamorphous systems in sustained release and combination therapy for insoluble small molecules. International Journal of Pharmacy and Pharmaceutical Sciences, 7(1), pp. 385–391.
17. Amara RO, Bendala NM. Pre-formulation solubility study of praziquantel in different media and solubilizing agents using the saturation shake-flask method. Mediterranean Journal of Pharmacy and Pharmaceutical Sciences. 2025 Jan 20(AheadOfPrint):75-81.
18. Javed S, Mangla B, Almoshari Y, Sultan MH, Ahsan W. Nanostructured lipid carrier system: A compendium of their formulation development approaches, optimization strategies by quality by design, and recent applications in drug delivery. Nanotechnology reviews. 2022 Apr 22;11(1):1744-77.
19. Awadeen RH, Boughdady MF, Meshali MM. Quality by design approach for preparation of zolmitriptan/chitosan nanostructured lipid carrier particles–formulation and pharmacodynamic assessment. International Journal of Nanomedicine. 2020 Nov 2:8553-68.
20. Yadav R, Mani RJ, Sharma A, Kumar A, Katare D. Formulation and optimization of metformin-berberine loaded solid lipid nanoparticles for their neuroprotective effects in the brain. Journal of Applied Pharmaceutical Research. 2025 Oct 31;13(5):114-33.
21. Deng J, Wu Z, Zhao Z, Wu C, Yuan M, Su Z, Wang Y, Wang Z. Berberine-loaded nanostructured lipid carriers enhance the treatment of ulcerative colitis. International journal of nanomedicine. 2020 Jun 3:3937-51.
22. D'Souza S. A review of in vitro drug release test methods for nano-sized dosage forms. Advances in pharmaceutics. 2014;2014(1):304757.
23. Gheibi S, Kashfi K, Ghasemi A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomedicine & pharmacotherapy. 2017 Nov 1;95:605-13.
24. Johnson PD, Besselsen DG. Practical aspects of experimental design in animal research. ILAR journal. 2002 Jan 1;43(4):202-6.
25. Akhtar N, Akram M, Daniyal M, Ahmad S. Evaluation of antidiabetic activity of Ipomoea batatas L. extract in alloxan-induced diabetic rats. International journal of immunopathology and pharmacology. 2018 Nov;32:2058738418814678.
26. Abduallah AM, Ahmed AE, Bajaber MA, Alalwiat AA. Evaluation of the Antidiabetic Effects of Methanolic Extracts of Neem (Azadirachta indica) Seeds on the Streptozotocin-induced Wistar Rats. Pakistan Veterinary Journal. 2023 Oct 1;43(4).
27. Dong Z, Iqbal S, Zhao Z. Preparation of ergosterol-loaded nanostructured lipid carriers for enhancing oral bioavailability and antidiabetic nephropathy effects. Aaps Pharmscitech. 2020 Jan 13;21(2):64.
28. Dong Z, Iqbal S, Zhao Z. Preparation of ergosterol-loaded nanostructured lipid carriers for enhancing oral bioavailability and antidiabetic nephropathy effects. Aaps Pharmscitech. 2020 Jan 13;21(2):64.
29. Nie W, Jiang M, Li Y, Lan J, Li Z, Bi Z, Gu D, Zhang M, Ding Y, Zhang T. Zhimu-Huangbai codecoction for the treatment of type II diabetes mellitus through its self-assembling nanoparticles. Chinese Medicine. 2026 Jan 7;21(1):13.
30. Qushawy M. Effect of the surfactant and liquid lipid type in the physico-chemical characteristics of beeswax-based nanostructured lipid carrier (NLC) of metformin. Pharmaceutical Nanotechnology. 2021 Jun 1;9(3):200-9.

