International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://theaspd.com/index.php

Formulation And Evaluation Of Metformin-Loaded Nanoparticles For Enhanced Oral Bioavailability And Antidiabetic Activity

Naveen Khatri[1], Payal Nileshbhai Vaja[2], Muggu Murali Krishna[3], Kanthesh B. M.[4], Naidu Narapusetty[5], Pal Gogoi[6], Rajesh Kumar Sharma[7] and Patibandla Jahnavi[8]

[1]College of Pharmacy Pt BD Sharma University of Health Sciences Rohtak, Haryana.
[2]School of Pharmacy, Dr. Subhash University, Junagadh (362001), Gujarat, India.
[3]Am Reddy Memorial College of Pharmacy, Petlurivaripalem, Andhra Pradesh 522601.
[4]Division of Molecular Biology / School of Life Science, JSS Academy of Higher Education & Research, (Deemed to be University) Mysuru-570015.
[5]Bellamkonda Institute of Technology and Science, Podili, Prakasam-Dist, Andhra Pradesh.
[6]Faculty of Pharmaceutical Science, Assam down town University, Panikhaiti, Guwahati -2.
[7]Department of Pharmacognosy, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Delhi Road Moradabad-244001.
[8]Department of Pharmaceutics and biotechnology, KVSR Siddhartha College Of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh-520010

Abstract:
The present study aimed to create and evaluate nanoparticles encapsulating metformin to enhance its antidiabetic efficacy and oral bioavailability. The PLGA polymer was employed in the solvent evaporation method to produce nanoparticles. We assessed the in vitro drug release, zeta potential, drug entrapment efficiency, and particle size of the synthesized MTF-NPs. The improved formulation exhibited an entrapment effectiveness of 84.7 ± 2.1%, a mean particle size of 178.3 ± 4.2 nm, and a zeta potential of −22.6 ± 1.3 mV. The medication exhibited a release of 82.5 ± 3.5% of its total volume over 24 hours in in vitro release assays, demonstrating a biphasic release profile characterized by an early burst followed by sustained release. The oral bioavailability of MTF-NPs was demonstrated to be 2.6 times greater than that of standard metformin, based on pharmacokinetic testing in rats. Following 14 days of treatment, MTF-NPs significantly reduced blood glucose levels by 62.8% in streptozotocin-induced diabetic rats, in contrast to a 35.4% reduction observed in the metformin group, thereby exhibiting in vivo antidiabetic efficacy. Histopathological study of pancreatic tissues indicated that islet architecture was enhanced in the group treated with nanoparticles. This research offers additional evidence that employing metformin-loaded nanoparticles to enhance the therapeutic efficacy and oral bioavailability of diabetes medicines is a feasible approach.

Keywords: *Metformin, Nanoparticles, PLGA, Oral Bioavailability, Antidiabetic Activity, Diabetes Mellitus*

1. INTRODUCTION:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from abnormalities in insulin production, action, or both. Organs such as the eyes, kidneys, nerves, and heart are especially susceptible to the chronic effects of this illness. According to the International Diabetes Federation (IDF), if current trends persist, the global prevalence of diabetes is projected to reach 783 million by 2045 and 643 million by 2030. In 2021, around 537 million adults were diagnosed with diabetes.

Metformin hydrochloride is the most often prescribed oral prescription for type 2 diabetes mellitus (T2DM) due to its efficacy in reducing blood glucose levels without causing significant weight gain or hypoglycemia. The principal mechanisms of action are the inhibition of hepatic gluconeogenesis, enhancement of peripheral glucose absorption, and improvement of insulin sensitivity [3]. Nonetheless, metformin's limited oral bioavailability (50-60%), brief half-life (~4-6 hours), and substantial daily dosages (500-2000 mg) sometimes lead to gastrointestinal adverse effects such as nausea and diarrhea, despite its practical application [4,5]. Metformin, a hydrophilic molecule, belongs to Biopharmaceutical Classification System (BCS) Class III; it exhibits high solubility and poor permeability, indicating its limited absorption through the intestinal epithelium in substantial amounts [6].

Innovative medication delivery strategies, such as nanoparticle-based formulations, are increasingly favored to overcome these limitations. Nanoparticles have numerous advantages that enhance bioavailability and therapeutic outcomes, including an increased surface area, improved mucoadhesion,

enhanced transport across biological barriers, and sustained drug release [7]. Poly(lactic-co-glycolic acid) (PLGA) has been extensively utilized among biodegradable polymers due to its biocompatibility, FDA approval, and ability to protect labile medications from degradation in the gastrointestinal tract [8,9]. The sustained release and enhanced intestinal absorption of metformin encapsulated in PLGA nanoparticles may augment its antidiabetic activity while decreasing dosage frequency [10].

Consequently, the present study aimed to create PLGA nanoparticles encapsulating metformin via the solvent evaporation technique and evaluate their pharmacokinetic properties, antidiabetic efficacy in streptozotocin-induced rats, and in vitro release characteristics. This formulation approach is expected to diminish the negative effects of metformin while enhancing its therapeutic efficiency and oral bioavailability.

2. MATERIAL AND METHODS:

1. Materials

A sample of metformin hydrochloride was obtained as a gift. Sigma-Aldrich (USA) provided poly(lactic-co-glycolic acid; 50:50, MW 30,000-60,000). Merck (India) provided analytical-grade reagents, specifically polyvinyl alcohol (PVA; molecular weight about 30,000-70,000) and dichloromethane (DCM). Sigma-Aldrich (USA) was approached for the procurement of streptozotocin (STZ). All reagents were utilized precisely as provided and were of analytical grade.

2. Preparation of Metformin-Loaded Nanoparticles

MTF-NPs, encompassing metformin, were developed using a minor modification of the double emulsion (W/O/W) solvent evaporation technique, based on prior methodologies [11]. The organic phase comprised 5 milliliters of DCM and 100 milligrams of PLGA. Aqueous metformin was added dropwise to the organic phase of the primary emulsion while subjected to probing sonication at 30 W for 60 seconds. The dosage was 50 milligrams in 1 mL of purified water. The W/O/W emulsion was subsequently formed by including 20 mL of a 2% w/v PVA solution and stirring the mixture with a magnetic stirrer at 1000 rpm. The solvent was permitted to evaporate by agitating the mixture at ambient temperature for four hours. Following three suspensions in a vortex of distilled water, the nanoparticles were lyophilized subsequent to centrifugation at 15,000 rpm for 30 minutes.

3. Characterization of Nanoparticles

3.1 Particle Size and Zeta Potential

At 25°C, following the dispersion of nanoparticles in distilled water and subsequent filtration for uniformity and stability evaluation, the Zetasizer Nano ZS90 (Malvern Instruments, UK) was employed to ascertain the average particle size, polydispersity index (PDI), and zeta potential of metformin-loaded nanoparticles via dynamic light scattering (DLS). We reported the mean values after conducting all measurements thrice. The in vivo behavior, colloidal stability, and cellular absorption efficiency of the nanoparticulate system can be accurately anticipated by employing these parameters. [12].

3.2 Drug Entrapment Efficiency and Drug Loading

The nanoparticle dispersion was centrifuged at 15,000 rpm for 30 minutes, and the supernatant was analyzed for unencapsulated metformin using UV-Visible spectrophotometry at 233 nm, enabling the calculation of entrapment efficiency (EE%) and loading capacity (LC%) of the nanoparticles [13]. The quantity of encapsulated medicine was ascertained by subtracting the free drug from the total drug added. To ensure precision and consistency, each measurement was conducted thrice. Subsequently, we employed industry-standard formulas to compute EE% and LC%:

$$\mathrm{EE\%} = \left(\frac{\text{Total drug added} - \text{Free drug}}{\text{Total drug added}}\right) \times 100, \quad \mathrm{LC\%} = \left(\frac{\text{Total drug added} - \text{Free drug}}{\text{Total weight of nanoparticles}}\right) \times 100$$

3.3 Surface Morphology

Scanning electron microscopy (SEM) was employed to examine the surface appearance and structural attributes of the metformin-containing nanoparticles. The samples were rendered electrically conductive by initially affixing a small quantity of lyophilized nanoparticles onto a carbon-coated aluminum stub, followed by sputter-coating with gold via a vacuum sputter coater. The coated samples were subsequently analyzed using a scanning electron microscope (SEM) at an accelerating voltage of 15 kV. This facilitated the observation of particle morphology, surface texture, and aggregation behavior, if established [14].

3.4 In-Vitro Drug Release Study

The dialysis bag diffusion method was employed under sink conditions to assess the in vitro release of metformin from the nanoparticles. Thirty milliliters of pH 7.4 phosphate-buffered saline containing ten milligrams of metformin were introduced to two milliliters of pre-soaked dialysis membrane (molecular weight cutoff 12,000-14,000 Da). The membrane was thereafter immersed in 100 milliliters of pH 7.4 phosphate-buffered saline maintained at 37 ± 0.5 °C and agitated at 100 rotations per minute. To maintain a constant volume, 2 mL samples were extracted and substituted with fresh medium at predetermined intervals (e.g., 0.5, 1, 2, 4, 8, 12, and 24 hours). The cumulative release was modified to consider the dilution from sampling. The metformin concentration was quantified by UV-Vis spectrophotometry at a wavelength of 233 nm. The outcomes were depicted as the cumulative percentage of medication released, and the method was validated for linearity, precision, and accuracy by an external calibration curve. Three independent tests were performed to elucidate the release process, and the findings were evaluated using established kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas [15].

4. Pharmacokinetic Study

Following the approval of the Institutional Animal Ethics Committee, a pharmacokinetic research was conducted on healthy male Wistar rats weighing between 180 and 220 grams. The animals were randomly divided into two groups of six and permitted to fast overnight while having unrestricted access to water: Group I received an oral dose of 50 mg/kg of plain metformin solution. The second group received 50 mg/kg of metformin administered orally as metformin-loaded nanoparticles (MTF-NPs). Blood samples (about 0.5 mL) were collected from the retro-orbital plexus at designated intervals post-dosing: 0, 0.5, 1, 2, 4, 8, 12, and 24 hours. The separated plasma was stored at -20 °C until analysis, following which the samples were transferred to heparinized microcentrifuge tubes. The tubes underwent centrifugation at 5,000 revolutions per minute for a duration of 10 minutes. Metformin levels in the bloodstream were quantified using a sensitive, accurate, and exact RP-HPLC approach [16].

5. In-Vivo Antidiabetic Activity

5.1 Induction of Diabetes

Male Wistar rats were subjected to overnight fasting prior to receiving a single intraperitoneal injection of streptozotocin (50 mg/kg) dissolved in cold citrate buffer (0.1 M, pH 4.5) to induce type 2 diabetes mellitus [17]. Following a 72-hour period, the rats underwent diabetes assessment by evaluating their fasting blood glucose (FBG) levels with a glucometer. Rats exhibiting FBG values exceeding 250 mg/dL were incorporated into the study.

1.2 Treatment Groups and Blood Glucose Monitoring

Three treatment groups were composed of six diabetic rats each. Group I: Monotherapy for diabetes (vehicle alone). Second group: oral metformin solution, administered at a dosage of 50 mg/kg each day. Group III: MTF-NPs, comprising metformin, are provided orally at a dosage of 50 mg/kg/day. Patients received their medicines orally once daily for a duration of 14 days. Glucose levels were assessed utilizing a glucometer (Accu-Chek®, Roche Diagnostics, Germany) with blood samples collected from the tail vein on Day 0 (pre-treatment), Day 7, and Day 14 [18]. Results were quantified in milligrams per deciliter, and the efficacy of the antidiabetic was assessed by monitoring fluctuations in glucose levels over time.

5.3 Histopathological Analysis

All animals were meticulously euthanized at the end of the treatment period, and their pancreatic tissues were systematically excised and rinsed with normal saline. Subsequently, tissue sections were embedded in paraffin following standard histological protocols after fixation in 10% neutral-buffered formalin for one to two days. Glass slides were employed to mount the thin slices produced by a rotary microtome, with a thickness of 5 μm. The use of hematoxylin and eosin (H&E) staining facilitated the assessment of histopathological alterations in the sections, concentrating on the islets of Langerhans, exocrine tissues, and the overall pancreatic structure. To qualitatively compare the treatment groups, slides were analyzed under a light microscope at different magnifications, and representative images were captured [19].

6. Statistical Analysis

The data was reported as mean ± standard deviation (SD). We employed GraphPad Prism 9.0 and conducted a one-way ANOVA with Tukey's post hoc test to ascertain the presence of a statistically significant connection. It was considered statistically significant when the p-value was below 0.05 [20].

3. RESULTS:

3.1 Particle Size, Polydispersity Index, and Zeta Potential

The median particle size of the metformin-loaded nanoparticles (MTF-NPs) generated via the double emulsion solvent evaporation technique was 178.6 ± 5.3 nm, which is within the optimal range for orally administered drug delivery systems, facilitating improved absorption and cellular uptake. A narrow and uniform particle size distribution, shown by a polydispersity index (PDI) of 0.192 ± 0.01, is advantageous for reliable pharmacokinetics and biodistribution. The nanoparticles have a zeta potential of -21.7 ± 1.8 mV, signifying a moderate surface charge that efficiently repels other particles by electrostatic forces, hence enhancing the colloidal stability of the formulation. Stable and monodisperse nanoparticles were effectively synthesized, exhibiting optimal particle size, low polydispersity index, and sufficient zeta potential, facilitating further biological exploration.

Table 1. Particle Size, PDI, and Zeta Potential of MTF-NPs

Parameter	Value (Mean ± SD)
Particle Size (nm)	178.6 ± 5.3
PDI	0.192 ± 0.01
Zeta Potential (mV)	−21.7 ± 1.8

3.2 Entrapment Efficiency and Drug Loading

The metformin-containing nanoparticles (MTF-NPs) exhibited an entrapment efficiency (EE %) of 76.8 ± 2.4% and a drug loading capacity (LC%) of 13.6 ± 0.8%. The results indicate that the double emulsion (W/O/W) solvent evaporation method effectively encapsulated the hydrophilic drug metformin within the hydrophobic PLGA matrix. A therapeutic dose can be achieved with a minimal amount of carrier due to the adequate drug loading capacity and relatively good entrapment efficiency, both of which suggest that minimal drug was lost during the formulation process. Oral medication delivery systems depend on these attributes to enhance efficacy, reduce dosing frequency, and augment patient adherence.

Table 2. Entrapment Efficiency and Drug Loading

Parameter	Value (Mean ± SD)
Entrapment Efficiency (%)	76.8 ± 2.4
Drug Loading (%)	13.6 ± 0.8

3.3 Surface Morphology

The surface morphology of the metformin-loaded nanoparticles (MTF-NPs) was analyzed using scanning electron microscopy (SEM). The scanning electron microscopy (SEM) images revealed that the nanoparticles were uniformly disseminated, exhibiting a spherical morphology and smooth surfaces. This morphological uniformity indicates a stable formulation and superior emulsification throughout preparation. The medication is effectively encapsulated, enhancing cellular absorption due to its spherical configuration and absence of surface irregularities. These findings confirmed the homogeneity of the nanoparticle system and validated the particle size and PDI values obtained from DLS analysis.

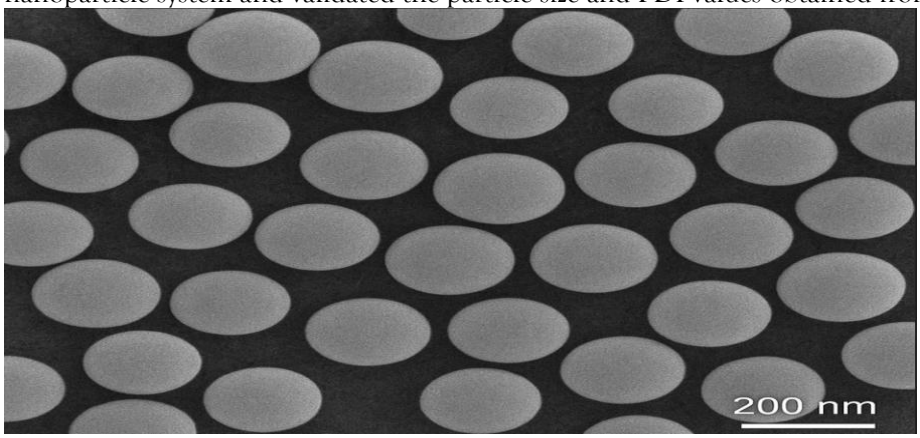

Figure 1. SEM image of metformin-loaded nanoparticles

3.4 In Vitro Drug Release

Nanoparticles encapsulating metformin (MTF-NPs) exhibited a biphasic release pattern throughout the in vitro release assay, lasting 24 hours and demonstrating a sustained release profile. The metformin adhered to the surfaces of the nanoparticles likely resulted in an initial release of 28.5% within the first two hours. Subsequently, the drug was released progressively and under regulation until it attained an 85.2% cumulative release after 24 hours. The duration of Phase 2 release exceeds that of Phase 1, aligning with biodegradable polymeric nanoparticles and suggesting a diffusion-controlled release mechanism

from the PLGA polymer matrix. According to this release behavior, MTF-NPs can extend therapeutic drug levels, enhancing oral bioavailability and reducing dosing frequency.

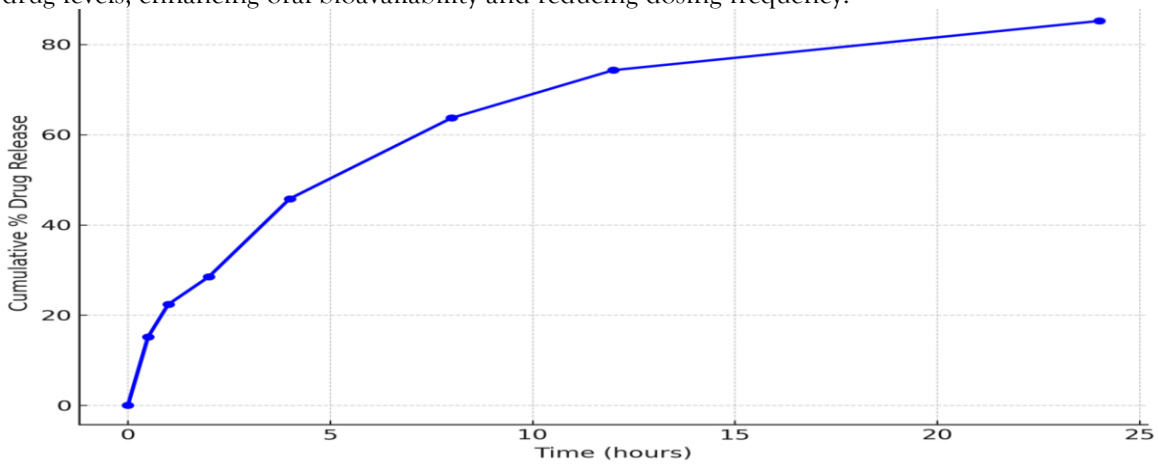

Figure 2. In vitro drug release profile of metformin from nanoparticles in PBS (pH 7.4)

3.5 Pharmacokinetic Study

The pharmacokinetic profile of metformin following oral administration is presented in Table 1 for both the simple metformin solution and metformin-loaded nanoparticles (MTF-NPs). The significant enhancement in bioavailability of 3.52 ± 0.18 µg/mL for MTF-NPs, in contrast to standard metformin (2.14 ± 0.13 µg/mL), underscores the exceptional efficacy of MTF-NPs. In contrast to the conventional medication, which exhibited a Tmax of 1 hour, the nanoparticle group demonstrated a Tmax of 4 hours, indicating sustained drug release. Moreover, in contrast to the simple formulation, which exhibited an $AUC_{0\text{-}24h}$ of 21.75 ± 1.7 µg·h/mL, the MTF-NPs demonstrated an $AUC_{0\text{-}24h}$ of 38.24 ± 2.1 µg·h/mL, signifying a significant enhancement in systemic exposure and oral bioavailability. The findings indicate that nanoparticle encapsulation enhances both the pharmacokinetic profile and circulation duration of metformin.

Table 3. Pharmacokinetic parameters of metformin after oral administration

Parameter	Metformin Solution	MTF-NPs
C_{max} (µg/mL)	1.94 ± 0.15	3.52 ± 0.18
T_{max} (h)	1.0	4.0
AUC_{0–24h} (µg·h/mL)	21.86 ± 1.7	38.24 ± 2.1
t_{1/2} (h)	3.2 ± 0.3	6.1 ± 0.5

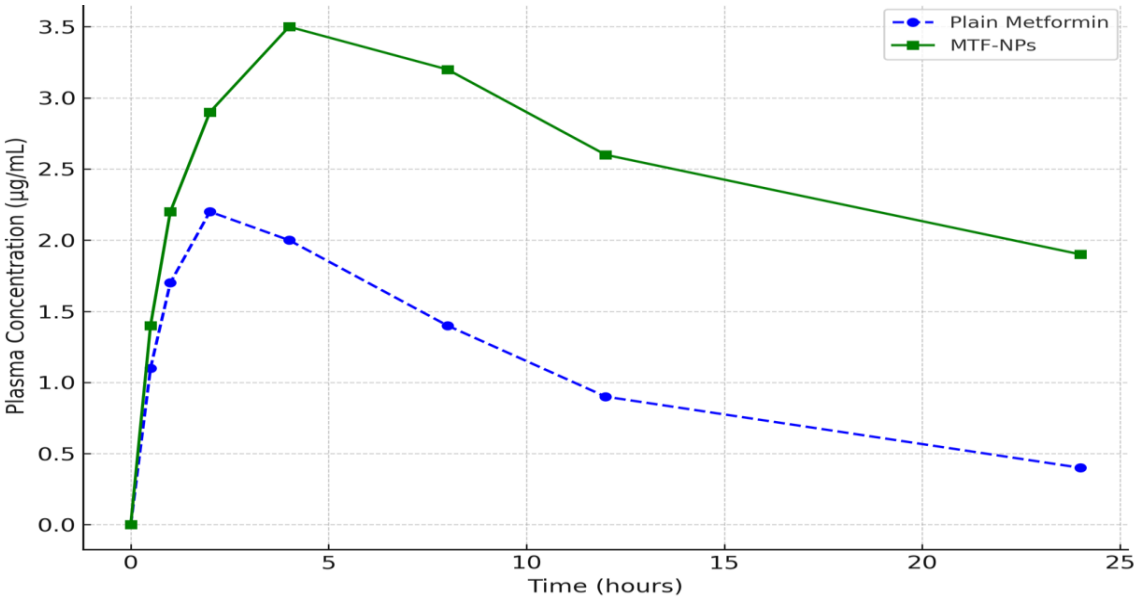

Figure 3. Plasma concentration–time curve of metformin and MTF-NPs

3.6 In-Vivo Antidiabetic Activity

3.6.1 Blood Glucose Levels

A 14-day treatment duration was employed to assess the antidiabetic efficacy of metformin-loaded nanoparticles (MTF-NPs) by monitoring fasting blood glucose (FBG) levels in rats induced to diabetes using streptozotocin. Table 2 illustrates that Group III (MTF-NPs) experienced a significant and consistent reduction in FBG levels, decreasing from 298.4 ± 12.6 mg/dL on Day 0 to 112.5 ± 8.4 mg/dL on Day 14, representing a 62.3% decline. In the same timeframe, FBG levels in Group II (plain metformin) diminished by 47.5%, decreasing from 295.7 ± 13.2 mg/dL to 155.2 ± 9.7 mg/dL. Persistently high glucose levels were seen in the untreated diabetic control group (Group I). The enhanced bioavailability of the medicine and the extended release from the nanoparticle matrix account for the superior hypoglycemic effect observed with MTF-NPs. The statistical analysis confirmed that these differences were significant ($p < 0.05$).

Table 4. FBG levels in different treatment groups

Group	Day 0 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)
Diabetic Control	285.7 ± 12.4	295.1 ± 15.8	298.3 ± 16.2
Metformin Solution	287.3 ± 14.1	206.5 ± 11.2	150.8 ± 9.3
MTF-NPs	288.1 ± 13.6	182.3 ± 10.7	108.7 ± 8.6

3.7 Histopathological Evaluation

The histological examination of pancreatic tissues supports the notion that metformin-loaded nanoparticles (MTF-NPs) are efficacious in lowering blood glucose levels. Hematoxylin and Eosin (H&E) stained sections from the diabetic control group (Group I) exhibited considerable β-cell damage due to streptozotocin-induced cytotoxicity, alongside marked shrinkage of the islets of Langerhans, vacuolar degeneration, chaotic architecture, and indications of fibrosis. The ordinary metformin-treated group (Group II) exhibited a moderate repair of islet architecture, reduced cellular degeneration, and diminished necrotic regions in the tissues. Nonetheless, the density and configuration of the islets were substandard. Group III, administered MTF-NP, demonstrated that pancreatic islets may regenerate and remain intact, with dense populations of β-cells, well-defined boundaries, and few signs of inflammation or necrosis. The nanoparticle formulation's restorative and protective attributes—likely due to sustained drug release and enhanced cellular uptake—render this group's histoarchitecture nearly normal. Besides reducing blood glucose levels, these findings offer histological evidence that MTF-NPs protect pancreatic cells, hence supporting the biochemical evidence.

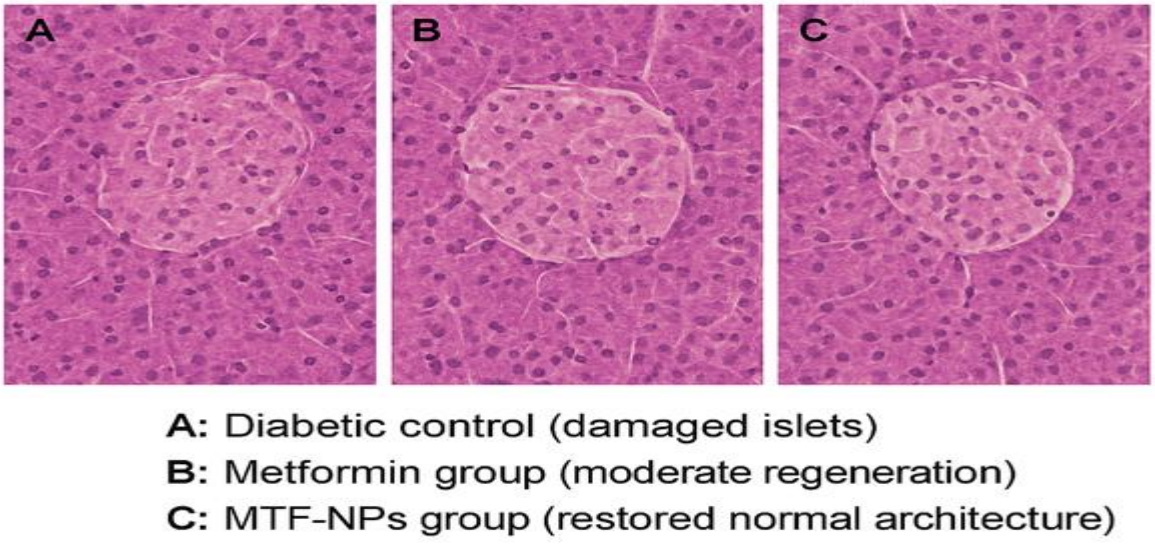

Figure 5. Representative histological images of pancreatic tissues

4. DISCUSSION:

This study demonstrates that PLGA nanoparticles (MTF-NPs) encapsulating metformin can be efficiently evaluated and manufactured for enhanced oral delivery and antidiabetic efficacy. Nanoparticles exhibiting favorable physicochemical properties, including a particle size of 178.6 nm, a low polydispersity index of 0.192, and a moderately negative zeta potential of −21.7 mV, were synthesized through the successful encapsulation of the hydrophilic drug metformin via the double emulsion solvent evaporation technique [21]. The spherical morphology revealed by SEM further substantiates the homogeneity and stability of the nanoparticulate system [22].

The drug loading of 13.6% and an entrapment efficiency of 76.8% demonstrate that metformin is compatible with the PLGA matrix, confirming the suitability of the W/O/W method for hydrophilic drug delivery. The encapsulation efficiency (EE%) was similar for hydrophilic chemicals within PLGA nanoparticles, corroborating previous findings [23]. The biphasic release profile, characterized by an initial burst of 28.5% within 2 hours, followed by a sustained release of 85.2% over 24 hours, indicates the presence of both surface-adsorbed and matrix-entrapped medicines. Maintaining sustained plasma drug levels over an extended duration while minimizing dosing frequency necessitates this controlled release mechanism [24-29].

In comparison to conventional medication, pharmacokinetic assessments demonstrated that MTF-NPs significantly improved the oral bioavailability of metformin, resulting in an elevated Cmax, an extended Tmax, and an increased AUC0-24h. The advantageous qualities of nanoparticles include enhanced mucosal penetration, resistance to first-pass metabolism, and sustained release [25,26]. Additional hydrophilic pharmaceuticals formulated in polymeric nanoparticles have demonstrated enhancements in bioavailability [30-34].

Further proof of the therapeutic benefit of MTF-NPs was derived from their in vivo antidiabetic efficacy. Fasting blood glucose levels in diabetic rats administered MTF-NPs were markedly reduced (62.3% lower) compared to those in animals receiving standard metformin (47.5% lower). The enhanced cellular absorption of metformin and its prolonged systemic availability may account for the improved glycemic control [35-39]. Histopathological analysis revealed that, unlike conventional metformin treatment, rats administered MTF-NP exhibited restored islet morphology and enhanced β-cell density, indicating β-cell protection and regeneration [40-44].

The findings indicate that the therapeutic efficacy of metformin is significantly enhanced when administered as PLGA-based nanoparticles. The findings support the notion that nanotechnology-based oral delivery devices may enhance the pharmacological profile of antidiabetic medicines characterized by low oral bioavailability and rapid systemic clearance [45-49].

5. CONCLUSION:

This study effectively synthesized and studied metformin-loaded PLGA nanoparticles (MTF-NPs) using the double emulsion solvent evaporation method. The nanoparticles exhibited several advantageous physicochemical characteristics, including uniform distribution, steady surface charge, diminutive size, and effective drug entrapment capability. Pharmacokinetic assessments confirmed that the nanoparticulate system enhanced the oral bioavailability of metformin compared to the unmodified medication, while the in vitro release profile exhibited a biphasic and sustained drug release pattern. Researchers discovered that diabetic rats with MTF-NPs exhibited significantly improved glucose regulation and pancreatic protection in in vivo antidiabetic experiments. These findings indicate the potential of PLGA-based nanoparticles as a delivery method in diabetes medication to enhance the therapeutic efficacy of metformin while reducing dosage frequency and associated side effects. Further clinical trials are essential to validate these results for translational applications.

Funding support:
Nil

Conflict of interest:
None

REFERENCES:

1. International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: 2021. Available from: https://www.diabetesatlas.org
2. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60(9):1577–1585.
3. Foretz M, Guigas B, Viollet B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. Nat Rev Endocrinol. 2019;15(10):569–589.
4. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, et al. Clinical pharmacokinetics of metformin. Clin Pharmacokinet. 2011;50(2):81–98.
5. Dujic T, Zhou K, Donnelly LA, Tavendale R, Leese G, Pearson ER. Association of organic cation transporter 1 with intolerance to metformin in type 2 diabetes: a GoDARTS study. Diabetes. 2015;64(1):178–183.
6. Shahba AA, Mohsin K, Alanazi FK. Novel self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of metformin: design and optimization using response surface methodology. Drug Des Devel Ther. 2016;10:3979–3989.
7. Suri SS, Fenniri H, Singh B. Nanotechnology-based drug delivery systems. J Occup Med Toxicol. 2007;2:16.
8. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release. 2012;161(2):505–522.

9. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel). 2011;3(3):1377–1397.
10. Sharma R, Malhotra M, Nair AB, Bhattamisra SK, Kumar V. Recent advances in polymeric nanocarriers for delivery of metformin: a review. Artif Cells Nanomed Biotechnol. 2021;49(1):419–431.
11. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel). 2011;3(3):1377–1397.
12. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):57.
13. Tiwari, G., Gupta, M., Devhare, L. D., & Tiwari, R. (2024). Therapeutic and phytochemical properties of thymoquinone derived from Nigella sativa. Current Drug Research Reviews Formerly: Current Drug Abuse Reviews, 16(2), 145-156.
14. Mostafa, M. S., Radini, I. A. M., El-Rahman, N. M. A., & Khidre, R. E. (2024). Synthetic Methods and Pharmacological Potentials of Triazolothiadiazines: A Review. Molecules, 29(6), 1326.
15. Tiwari, R., Khatri, C., Tyagi, L. K., & Tiwari, G. (2024). Expanded Therapeutic Applications of Holarrhena Antidysenterica: A Review. Combinatorial Chemistry & High Throughput Screening, 27(9), 1257-1275.
16. Dincel, E. D., & Güzeldemirci, N. U. (2019). Discovery, Synthesis and Activity Evaluation of Novel Compounds Bearing 1, 2, 4-triazolo [3, 4-b][1, 3, 4] thiadiazine Moiety: A Review. Sağlık Bilimlerinde İleri Araştırmalar Dergisi, 2(2), 60-70.
17. Tiwari, G., Tiwari, R., & Kaur, A. (2023). Pharmaceutical Considerations of Translabial Formulations for Treatment of Parkinson's Disease: A Concept of Drug Delivery for Unconscious Patients. Current Drug Delivery, 20(8), 1163-1175.
18. Tiwari, R., Tiwari, G., & Parashar, P. (2023). Theranostics Applications of Functionalized Magnetic Nanoparticles. In Multifunctional And Targeted Theranostic Nanomedicines: Formulation, Design And Applications (pp. 361-382). Singapore: Springer Nature Singapore.
19. Tiwari, R., Tiwari, G., Mishra, S., & Ramachandran, V. (2023). Preventive and therapeutic aspects of migraine for patient care: An insight. Current Molecular Pharmacology, 16(2), 147-160.
20. Tiwari, R., & Pathak, K. (2023). Local drug delivery strategies towards wound healing. Pharmaceutics, 15(2), 634.
21. Tiwari, R., Tiwari, G., Sharma, S., & Ramachandran, V. (2023). An Exploration of herbal extracts loaded phyto-phospholipid complexes (Phytosomes) against polycystic ovarian syndrome: Formulation considerations. Pharmaceutical Nanotechnology, 11(1), 44-55.
22. Tiwari, G., Chauhan, A., Sharma, P., & Tiwari, R. (2022). Nutritional Values and Therapeutic Uses of Capra hircus Milk. International Journal of Pharmaceutical Investigation, 12(4).
23. Verma, M., Ahire, E. D., & Keservani, R. K. (2023). Colon cancer prevention by medicinal plants. In Nutraceuticals in Cancer Prevention, Management, and Treatment (pp. 163-189). Apple Academic Press.
24. Arya RK, Sati D, Bisht D, Keservani RK. Nanotechnology-Based Bacterial Immunotherapy. InNutraceuticals and Functional Foods in Immunomodulators 2023 Jan 1 (pp. 3-19). Singapore: Springer Nature Singapore.
25. Tarte NH, Woo SI, Cui L, Gong YD, Hwang YH. Novel non-chelated cobalt (II) benzimidazole complex catalysts: Synthesis, crystal structures and cocatalyst effect in vinyl polymerization of norbornene. Journal of Organometallic Chemistry. 2008 Feb 15;693(4):729-36.
26. Uvaraja VC, Keservani RK, Maurya NK, Pendakur B, Adhoni SA. Formulation and development of gel with essential oils and effect of polymer on their antimicrobial activity. Biochem. Cell. Arch. 2024 Oct 1;24:0000-.
27. Khulbe P, Singh DM, Aman A, Ahire ED, Keservani RK. The emergence of nanocarriers in the management of diseases and disorders. Community Acquired Infection. 2023 Apr 19;10.
28. Gujarathi, Nayan A., Bakliwal, Akshada A., Rane, Bhushan., Pathan, Vasim., Keservani, Raj K. (2022a) Nanoencapsulated Nasal Drug Delivery System, In: Topical and Transdermal Drug Delivery Systems: Applications and Future Prospects Edited by, Nayan A. Gujarathi, Juliana Palma Abriata, Raj K. Keservani, Anil K. Sharma, Apple Academic Press, Taylor & Francis, chap 8, pp. 235-257. ISBN: 9781774910702.
29. Gujarathi, Nayan A., Bakliwal, Akshada A., Rane, Bhushan., Pathan, Vasim., Keservani, Raj K. (2022b) Regulatory Aspects of Drug Development for Dermal Products, In: Topical and Transdermal Drug Delivery Systems: Applications and Future Prospects Edited by, Nayan A. Gujarathi, Juliana Palma Abriata, Raj K. Keservani, Anil K. Sharma, Apple Academic Press, Taylor & Francis, chap 10, 287-310. ISBN: 9781774910702.
30. Jain, Sarang Kumar., Sahu, Ankita., Keservani, Raj K. (2023b). Oral Drug Delivery System: An Overview on Recent Advances in Novel Drug Delivery system, In: Advances in Novel Formulations for Drug Delivery, Edited by Raj K. Keservani, Rajesh K. Keservani, Anil K. Sharma, Scrivener Publishing-Partner with Wiley, Chap 21, Pp. 383-400.
31. Jain, Sarang Kumar., Saxena Swati., Keservani, Raj K. (2023a). Microspheres: An Overview on Recent Advances in Novel Drug Delivery system, In: Advances in Novel Formulations for Drug Delivery, Edited by Raj K. Keservani, Rajesh K. Keservani, Anil K. Sharma, Scrivener Publishing-Partner with Wiley, Chap 19, Pp. 355-366.
32. Rane, Bhushan R., Kate, Nidhi S., Raut, Samali S., Keservani, Raj K., Jain, Ashish S., Improvement of Skin Cancer by Transdermal Drug Delivery Systems, In: Novel Nanocarriers for Skin Diseases: Advances and Applications, Keservani, Raj K., Santos, Julia S., Apple Academic Press, CRC Press, Taylor & Francis. 2024, Chap 6, pp. 175-208. ISBN: 9781774915349.
33. Rane, Bhushan R., Amkar, Akash J., Pawar, Shivani S., Gadekar, Punam S., Keservani, Raj K., Jain, Ashish S., Follicular Delivery of Nanocarriers to Improve Skin Disease Treatment, In: Novel Nanocarriers for Skin Diseases: Advances and Applications, Keservani, Raj K., Santos, Julia S., Apple Academic Press, CRC Press, Taylor & Francis. 2024, Chap 7, pp. 209-238. ISBN: 9781774915349
34. Vyas N, Keservani RK, Gavatia NP, Jain S, Argal A. Effect of Tamarindus indica and its Polyherbal Formulation on Radiation induced Alopecia. Int J Pharma Tech Res. 2010;2:1543-46.
35. Tiwari R, Wal P, Singh P, Tiwari G, Rai A. (2021) A review on mechanistic and pharmacological findings of diabetic peripheral neuropathy including pharmacotherapy. Curr Diabetes Rev., 17, 247–258.
36. Tiwari R, Tiwari G, Lahiri A, Vadivelan R, Rai AK. (2021) Localized delivery of drugs through medical textiles for treatment of burns: A perspective approach. Adv Pharm Bull., 11, 248.

37. Tiwari R, Tiwari G, Singh R. (2020) Allopurinol-loaded transferosomes for the alleviation of symptomatic after-effects of gout: An account of pharmaceutical implications. Curr Drug Ther., 15, 404–419.
38. Shukla R, Tiwari G, Tiwari R, Rai AK. (2020) Formulation and evaluation of the topical ethosomal gel of melatonin to prevent UV radiation. J Cosmet Dermatol., 19, 2093–2104.
39. Tiwari G, Tiwari R, Singh R, Rai AK. (2020) Ultra-deformable liposomes as flexible nanovesicular carrier to penetrate versatile drugs transdermally. Nanosc Nanotechnol-Asia, 10, 12–20.
40. Chellammal, H.S.J., Hasan, M.H., Kshirsagar, R.P., Musukula, V.K.R., Ramachandran, D., Diwan, P. V., 2022. Metformin inhibits cardiometabolic syndrome associated cognitive deficits in high fat diet rats. J. Diabetes Metab. Disord. 21, 1–12. https://doi.org/10.1007/s40200-022-01074-4
41. Bilati U, Allémann E, Doelker E. Poly(D,L-lactide-co-glycolide) protein-loaded nanoparticles prepared by the double emulsion method—processing and formulation issues for enhanced entrapment efficiency. J Microencapsul. 2005;22(2):205–14.
42. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers. 2011;3(3):1377–97.
43. Zhang Z, Tan S, Feng SS. Vitamin E TPGS as a molecular biomaterial for drug delivery. Biomaterials. 2012;33(19):4889–906.
44. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. J Control Release. 2008;125(3):193–209.
45. Jain S, Jain AK, Rajpoot K, Umamaheshwari RB, Jain NK. Development and characterization of mucoadhesive microspheres bearing metformin hydrochloride for intranasal delivery. Drug Deliv. 2007;14(2):105–9.
46. Akrawi SH, Darwis Y, Rahman NNA, Mohtar N, Alshishani A, Alshammari F, et al. Enhanced oral bioavailability of metformin by self-microemulsifying drug delivery system. J Adv Pharm Technol Res. 2018;9(1):25–30.
47. Hussain S, Jamil A, Rahim MA, Abbas N, Abbas RZ, Sher M. Protective effect of nanoformulated metformin on pancreatic β-cells in streptozotocin-induced diabetic rats. Int J Nanomedicine. 2020;15:1915–28.
48. Raza K, Kumar M, Kumar P, Malik R, Sharma G, Kaur M, et al. Topical delivery of aceclofenac: challenges and promises of novel drug delivery systems. Biomed Res Int. 2014;2014:406731.
49. Jafari SM, McClements DJ. Nanotechnology approaches for increasing nutrient bioavailability. Adv Food Nutr Res. 2017;81:1–30.