

Formulation by Ether Injection Method and In-Vitro, In-Vivo Evaluation of Tween 80 Based Metformin Loaded Niosomes: A Smart Strategy in the Drug Delivery System

Md. Mahbubol Alam^{1,2,*}, Fahamida Binta Anower^{1,3}, Kazi Nadia Haque Sharna³, Tasin Ahmed Fahim², Md. Minhajul Alam², Eity Akter³ & Tania Ferdous Urmi³

¹Department of Pharmacy, Faculty of Life and Earth Sciences, Jagannath University (JnU), Dhaka-1100, Bangladesh. ²Department of Pharmacy, Faculty of Science and Engineering, University of Information Technology and Sciences (UITS), Dhaka-1212, Bangladesh. ³Department of Pharmacy, Faculty of Science, Engineering and Technology, Bangladesh University (BU), Dhaka-1207, Bangladesh. Corresponding Author (Md. Mahbubol Alam) Email: mahbubhasan089@gmail.com*



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ABSTRACT

Metformin hydrochloride is one of the first-line oral antidiabetics but because of low oral bioavailability and biological half-life, there is need for formulation of more advanced drug delivery systems for this drug. In this study, niosomes loaded with metformin were formulated by the use of ether injection technique to deliver the drug by controlling the release and increasing its antidiabetic activity. Different formulations (F1-F5) were formulated with varying ratios of Tween 80 to cholesterol and these formulations were evaluated by their calibration curve, drug release study, and in vivo antidiabetic study using alloxan induced diabetic Swiss albino mice. The results of the drug release study show that formulations released varying amounts of the drug depending on the surfactant concentration with formulation F3 releasing maximum drug amount (74.67%) within 120 min. As far as in vivo study is concerned, all formulations significantly decreased blood glucose level in comparison with diabetic control group. From among all the formulations, formulation F2 was found to exhibit maximum glucose lowering activity (63.15%) followed by formulation F1 (62.31%), while standard group with metformin treatment showed decrease in glucose levels (75.70%). The above-mentioned results clearly indicate that MTH loaded niosomes are efficacious in reducing diabetes in a controlled manner but further studies on pharmacokinetics and bioavailability are needed.

Keywords: Alloxan-Induced Diabetes; Antidiabetic Activity; Cholesterol; Controlled Drug Release; Metformin Hydrochloride; Nanocarrier; Niosomes; Oral Drug Delivery; Plasma Glucose; Tween.

1.0. Introduction

Metformin Hydrochloride continues to be the most popular first-line oral antihyperglycemic drug for treatment of Type 2 Diabetes Mellitus (T2DM). Metformin belongs to the group of biguanides and is used extensively due to the proven efficiency in blood glucose lowering without leading to hypoglycemia or gaining in body weight [1]. Furthermore, the drug has been found to lower the risks of complications associated with diabetes and cardiovascular diseases; hence it becomes the best choice for patients with T2DM. Regardless of all those advantages, metformin has relatively poor oral bioavailability of around 50–60%, since it is poorly absorbed in the intestines, and the drug is considered BCS Class III drug [2]. Besides that, relatively short half-life of metformin forces it to be administered repeatedly, which negatively affects the patient compliance and therapeutic effect [3].

In order to overcome problems of traditional dosage form, drug delivery systems based on nanotechnology gained popularity. Among other technologies, niosomes are especially interesting because of their ability to encapsulate both hydrophilic and lipophilic drugs, to increase drug stability and oral bioavailability, and to provide controlled and targeted drug delivery [4]. Niosomes are the nano-size vesicles consisting of non-ionic surfactants and cholesterol, forming bilayer membrane protecting drugs from decomposition and increasing circulating time. Compared to liposomes, niosomes provide more stable conditions, cheaper production, and easier storage [5].

Various studies have confirmed the usefulness of niosomal drug delivery systems in improving the effectiveness of poorly absorbed drugs through improved dissolution properties, controlling drug release, and prolonging gastric

retention time. The use of metformin in niosomal vesicles has therefore been suggested as an appropriate method of enhancing its pharmacokinetics profile while ensuring maintenance of plasma drug concentration. Such a strategy will not only reduce the dosing regimen but also minimize gastrointestinal side effects in order to ultimately ensure good compliance with treatment [6].

Considering these facts, the current research work has been designed to prepare metformin-containing niosomes using ether injection technique and to investigate in vitro release characteristics and in vivo antidiabetic activity of such niosomes. Various formulations having different ratios of Tween 80 and cholesterol have been prepared in order to see the influence of the change in composition on drug release pattern and glucose lowering effect. It is expected that the findings of this study will help in developing an efficient controlled release oral drug delivery system for metformin.

1.1. Objectives of the Study

The objectives of this current study can be stated as follows:

- 1) Preparation of niosomes loaded with metformin hydrochloride using the method of ether injection with different ratios of Tween 80 and cholesterol.
- 2) Design and optimization of different formulations of niosomes (F1-F5) for controlled drug delivery through oral route.
- 3) Development of a calibration curve of metformin hydrochloride for quantitative analysis by UV-visible spectrophotometry.
- 4) Determination of in vitro drug release pattern of the formulations by membrane diffusion technique.
- 5) Evaluation of in vivo antidiabetic activity of the formulations against induced diabetic conditions in Swiss albino mice by monitoring changes in plasma glucose concentration.
- 6) Comparison of drug release pattern and antihyperglycemic activity among different formulations to determine the best formulation among them.
- 7) Examination of possibilities of niosomal encapsulation as a controlled release drug delivery vehicle to improve the efficacy of metformin hydrochloride.

2.0. Literature Review

Numerous researches have shown that niosomes provide the opportunity to increase the efficiency of medications by improving their stability, increasing the circulating time, preventing biological degradation, and providing the controlled drug release. Incorporation of metformin into niosomal vesicles is considered as a way of solving poor intestinal absorption and bioavailability problems [7].

Past research efforts have revealed that formulations of metformin-loaded niosomes have been successfully prepared by using different methods such as the ether injection method. These past research works have demonstrated that formulation parameters like the nature of surfactants used, the surfactant-to-cholesterol ratio, and

the conditions of preparation have a profound effect on vesicle formation, drug entrapment, and drug release. Increased amounts of non-ionic surfactants have been found to change vesicle size and permeability to influence the rate and extent of drug release. Optimization of formulation parameters is therefore vital in preparing an effective niosomal delivery system for metformin hydrochloride [8].

From the above review, it can be concluded that niosomal encapsulation could serve as an effective approach in increasing oral drug delivery efficiency of metformin hydrochloride. However, further experimental studies are required to optimize the formulation parameters and pharmacological performance of these preparations. Therefore, the purpose of the current research effort is to prepare metformin hydrochloride loaded niosomes by using the ether injection method and to assess their controlled release profile and antidiabetic effect in alloxan induced diabetic mice.

3.0. Materials and Methods

3.1. Materials and Equipments

Chloroform, cholesterol, Tween 80, and alloxan were gathered from Bangladesh University's laboratory. Metformin, the standard drug, was acquired from Opsonin Pharma Limited in Bangladesh. Analytical-grade chemicals were used for all the other chemicals, and distilled water was used for the whole project. The study utilized a dissolution test apparatus (Model: VDA 8D, Veego, India), a pH meter (Model: AD 1200, Romania), a UV-Visible Spectrophotometer (Model: T60, PG Instruments, India), an electronic analytical balance (Model: DHB $\pm$ 1, T-Scale, Taiwan), and a sonicator bath (Model: Powersonic 603, Hwashin Technology, Korea).

3.2. Experimental Animals

Albino Swiss mice (*Mus musculus*) of either gender, with body weights of 25-35 g and ages of 6-8 weeks were used for the analgesic activity test. These mice were procured from an authorized laboratory animal breeder and made available through the University of Jahangirnagar, Savar, Dhaka, Bangladesh. They were kept in conventional polypropylene cages under regulated laboratory conditions, i.e., temperature $25 \pm 2^{\circ}\text{C}$, relative humidity 55-65% and 12 hours light/dark photoperiods. Mice had free access to food and water. Acclimatization to the laboratory environment lasted for one week prior to experiments [9].

3.3. Preparation of Metformin Loaded Niosomes

Metformin hydrochloride-loaded niosomes were prepared using the Ether Injection Method. Specific amounts (200 mg) of metformin hydrochloride, Tween 80, and cholesterol were accurately weighed and dissolved according to the formula preparation procedure [6]. The mixture of surfactant and cholesterol was dissolved in chloroform, and the organic phase was slowly injected into an aqueous phase preheated to 60°C . When the organic solvent evaporated, vesicles were formed automatically, producing metformin-loaded niosomal suspensions. The preparations were then further processed using sonication in order to decrease the size of vesicles and obtain a homogeneous vesicular dispersion. Five formulations (F1-F5) were prepared by changing the amount of Tween 80 while keeping the amounts of metformin hydrochloride (200 mg) and cholesterol (200 mg) constant [10]. The method of operation consists of the subsequent steps.

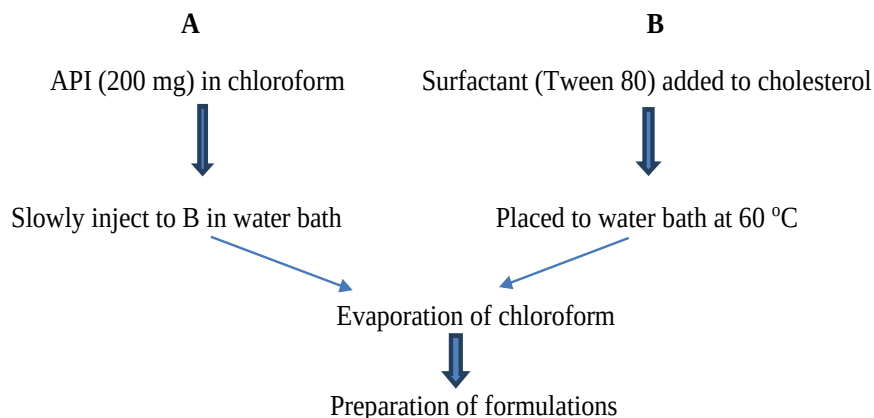


Figure 1. Flowchart of Ether Injection Method to Prepare Metformin Loaded Niosomes.

3.4. Standard Calibration Curve of Metformin

For constructing the standard curve, an aliquot of purified metformin HCl weighing 10 mg was added to phosphate buffer and the final volume was fixed at 100 mL within a laboratory volumetric flask. After filtration, a 10 mL filtrate aliquot was placed in a laboratory volumetric container (100 mL) and then diluted to obtain a standard solution with a concentration of 10 µg/mL. The solutions at varying concentrations (1, 3, 5, 7, 9 µg/mL) were prepared through proper dilution and the absorbance were observed at max 232 nm using UV-visible spectrophotometer. The absorbance was positioned against their corresponding concentration to generate the calibration curve. The graph was used to derive the linearity equation for subsequent calculations [6].

3.5. Drug Release Study (In-vitro)

The pattern of in vitro release of a batch of five metformin based niosomal formulations was studied using the technique of membrane driven diffusion for 2 hours. The release profiles of the formulations were assessed using egg membranes as a biological barrier [11]. To prepare egg membranes, egg shells were kept in the solution of 20 % acetic acid. After 24 hours egg membranes were separated from egg shells. Then membranes were collected and washed out for dissolution test. Each egg membrane, acting as a donor compartment, was loaded with niosomal formulation providing 200 mg metformin-equivalent dose [12]. In the USP type II dissolution apparatus (paddle method), the membrane was placed in Phosphate buffer (900 mL) maintained at around 37 °C and adjusted to pH 6.8 with 1 M sodium hydroxide, functioning as the receptor compartment. The paddle speed was fixed at 100 rpm and sustained for 120 minutes [13]. During the study, 10 mL test samples were pipetted at 15-minute intervals up to 120 minutes using micropipettes, with the withdrawn volume replaced by fresh medium to sustain sink conditions. Each collected sample underwent filtration through a Whatman filter, followed by dilution [14]. UV-visible spectrophotometric analysis was performed at 232 nm (λ_{max}) to measure the absorbances of the samples, employing phosphate buffer adjusted to pH 6.8 as the blank [6]. The percentage value of drug released from each formulation was calculated using Microsoft Excel.

3.6. Anti-diabetic Activity Test

Mice were given an injection of alloxan monohydrate (90 mg/kg) dissolved in saline (100 µL/mouse) through the peritoneal (i.p.) to induce diabetes after a 16-hour fast [15]. Following a 48-hour period, plasma glucose levels

were assessed using an OK meter (Match glucometer) from Hsinchu, Taiwan, utilizing a sample of blood drawn from the mice's tail vein. Mice exhibiting blood sugar levels exceeding 8.5-11.5 mmol/L are classified as diabetic. Healthy mice of the same age were utilized to investigate the effects of the extract on normal mice [16].

4.0. Result and Discussion

4.1. Preparation of Metformin Hydrochloride Niosomes

Five metformin hydrochloride-niosomes (F1-F5) were effectively prepared by employing the ether injection method (Figure 1), where the amount of Tween 80 was varied, but the amounts of metformin hydrochloride and cholesterol were kept the same. Each formulation was coded as indication in Table 1. In order to investigate the effect of surfactant concentration on the formulations, standard calibration, drug release, and antidiabetic experiments were conducted.

Table 1. Composition of Metformin Hydrochloride -Loaded Niosomal Formulations

Prepared by the Ether Injection Method.

Formulation Code	Metformin (mg)	Tween 80 (mL)	Cholesterol (mL)	Metformin: Surfactant: Cholesterol	Chloroform (mL)	Total Volume (mL)
F-1	200	200	200	1:1:1	20	600
F-2	200	300	200	1:1.5:1	20	700
F-3	200	400	200	1:2:1	20	800
F-4	200	500	200	1:2.5:1	20	900
F-5	200	600	200	1:3:1	20	1000

4.2. Preparation of Standard Calibration Curve

Calibration curve for metformin hydrochloride was prepared using UV-Vis spectrophotometer at 232 nm. Calibration curve showed very good linearity with regression equation $y = 0.0165x + 0.1421$ and R^2 value of 0.9946. Thus, it can be concluded that this analytical method can be used for quantification of metformin in the dissolution test. R^2 value shows good correlation between concentration and absorbance of the solution. The curve, shown in Figure 2, was utilized for additional drug assessment because it demonstrated strong linearity.

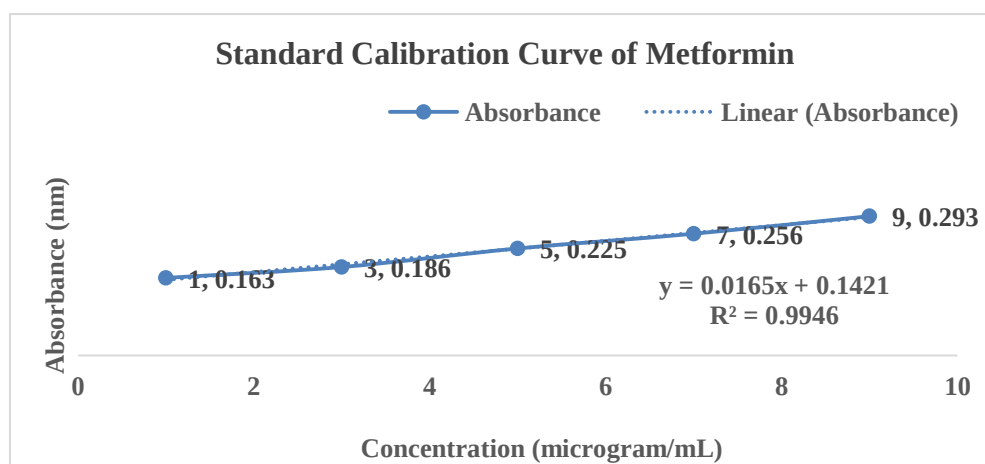


Figure 2. Standard Calibration Curve of Metformin Hydrochloride.

4.3. In Vitro Drug Release Study

The release pattern of metformin hydrochloride for the five preparations revealed a significant influence of the Tween 80 concentration on release properties. Table 2 represents the percent of drug release and Figure 3 illustrates the drug release profiles of the five formulations and helps to choose the optimized formulation. The cumulative releases at 120 minutes were 51.76% (F1), 56.66% (F2), 74.67% (F3), 52.30% (F4), and 44.67% (F5). Of the five preparations, F3 was found to have the maximum cumulative release of drug, which means that the ratio of the Tween 80-to-cholesterol used in this preparation allows diffusion of metformin through the membrane effectively. On the contrary, F5, with the maximum amount of surfactant, had the least drug release, implying that excess amount of surfactant leads to greater bilayer rigidity and stability of vesicles and, hence, slower diffusion of the drug.

Table 2. In vitro Cumulative Percentage of Drug Release Profiles of Metformin Hydrochloride-Loaded Niosomal Formulations (F1-F5).

Formulation	Time(min)							
	15	30	45	60	75	90	105	120
F1	3.76	9.76	17.4	21.76	25.58	29.94	44.67	51.76
F2	5.4	10.3	16.85	21.76	29.4	43.58	52.30	56.66
F3	1.58	2.67	12.49	21.21	29.4	44.67	67.58	74.67
F4	11.94	19.58	21.21	23.4	27.21	38.67	45.76	52.3
F5	5.94	8.67	10.85	17.94	31.03	43.03	44.12	44.67

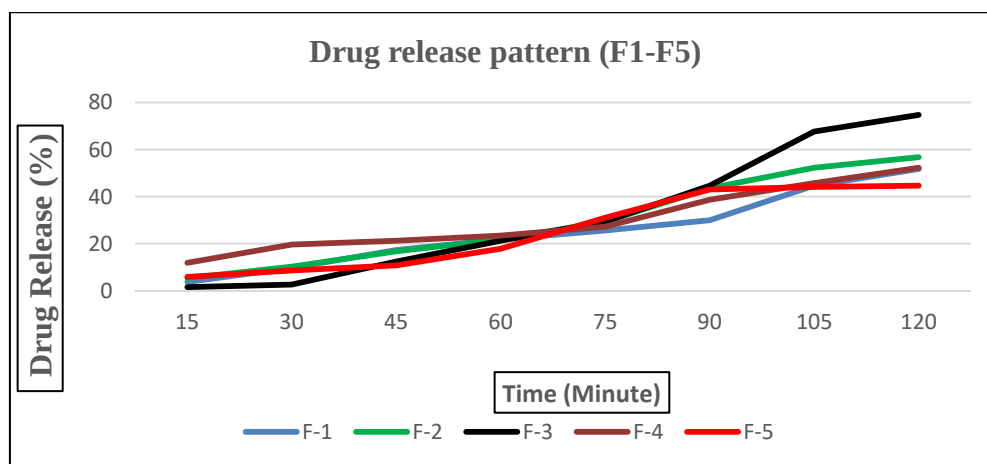


Figure 3. Comparative Percentage of Drug Release Profiles of Metformin Hydrochloride-Loaded Niosomal Formulations (F1-F5).

4.4. In Vivo Antidiabetic Activity

The in vivo antidiabetic activity of the prepared formulations was studied in diabetic Swiss albino mice induced using alloxan by measuring the levels of plasma glucose for a period of 240 minutes. All formulations showed a gradual reduction in blood glucose as compared to the diabetic control group, showing the efficacy of antihyperglycemic activity of the formulations. The antihyperglycemic activity of the niosomal formulation is summarized in Table 3.

Table 3. Plasma Glucose Levels (mmol/L) Following Administration of Metformin Hydrochloride-Loaded Niosomal Formulations in Alloxan-Induced Diabetic Mice.

Group	0 min	60 min	120 min	180 min	240 min
Control	24.75	24.03	22.65	21.68	20.94
Standard	26.35	21.88	18.81	14.65	10.67
F1	23.82	20.46	17.35	15.62	13.71
F2	24.44	21.24	18.61	16.92	14.10
F3	22.13	20.43	18.12	16.24	13.84
F4	24.91	22.68	19.83	17.21	15.41
F5	22.67	20.72	18.75	16.83	13.56

4.5. In Vivo Antidiabetic Activity

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Table 4. Plasma Glucose Levels (mmol/L) Following Administration of Metformin Hydrochloride-Loaded Niosomal Formulations in Alloxan-Induced Diabetic Mice.

Group	0 min	60 min	120 min	180 min	240 min
Control	24.75	24.03	22.65	21.68	20.94
Standard	26.35	21.88	18.81	14.65	10.67
F1	23.82	20.46	17.35	15.62	13.71
F2	24.44	21.24	18.61	16.92	14.10
F3	22.13	20.43	18.12	16.24	13.84
F4	24.91	22.68	19.83	17.21	15.41
F5	22.67	20.72	18.75	16.83	13.56

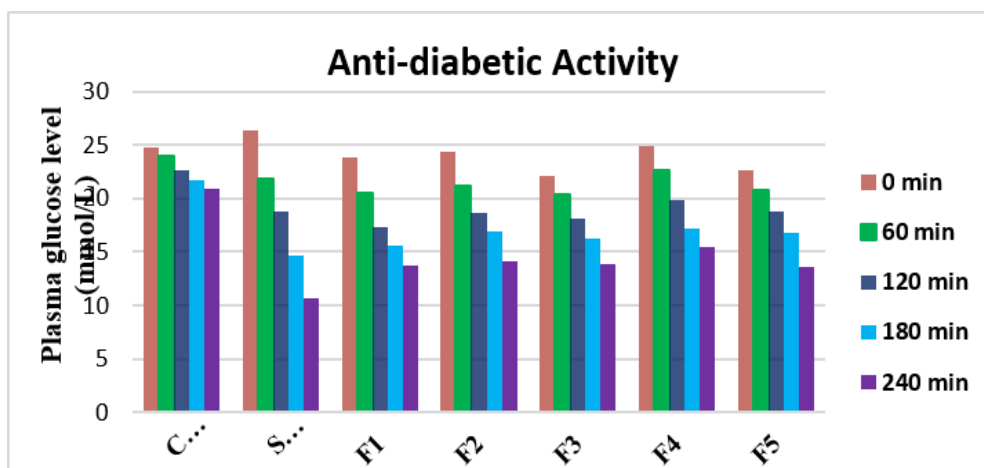


Figure 4. Effect of Metformin Hydrochloride-Loaded Niosomal Formulations on Plasma Glucose Levels in Alloxan-Induced Diabetic Mice.

According to Table 5 and Figure 5, it can be observed that the plasma glucose levels of the diabetic control group were higher than the plasma glucose levels of the treated groups after the treatment of alloxan. The plasma glucose levels increased after inducing diabetes and decreased after the administration of niosomal preparations. Mice treated with F1, F2, F3, F4, and F5 preparations at 10 mg/kg body weight had plasma glucose level reductions of 62.31%, 63.15%, 53.54%, 59.89%, and 58.18%, respectively, throughout the 240-minute period. Compared to the metformin group that showed a reduction in plasma glucose levels of 75.70%, preparation F2 exhibited maximum antihyperglycemic activity, followed by F1 preparation, as shown in Figure 5.

Table 5. Percentage Reduction in Plasma Glucose Levels Following Treatment with Metformin Hydrochloride-Loaded Niosomal Formulations.

Group	% reduction of plasma glucose levels
Control	0
Standard	75.70
F1	62.31
F2	63.15
F3	53.54
F4	59.89
F5	58.18

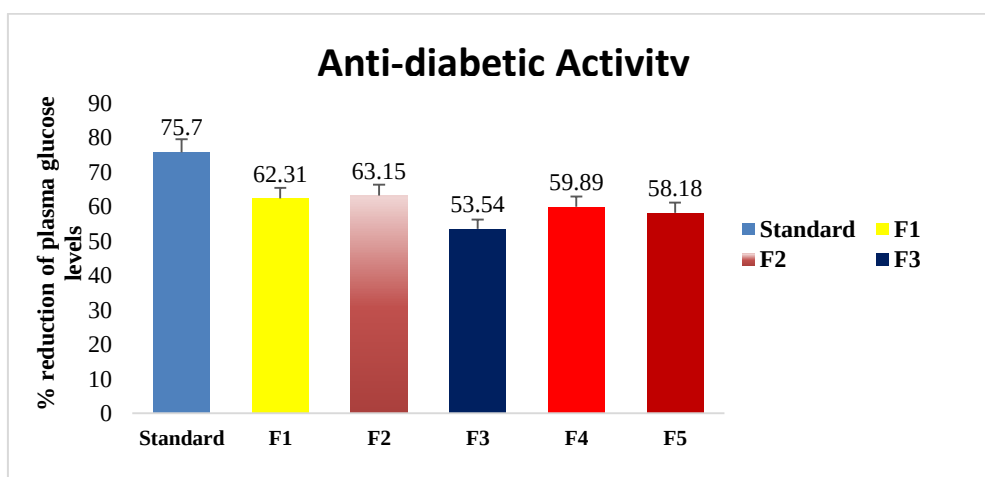


Figure 5. A Graphical Presentation of Percentage Reduction in Plasma Glucose Levels After Treatment with Metformin Hydrochloride-Loaded Niosomal Formulations.

4.6. Overall Discussion

In general, the data obtained suggests that physicochemical properties of niosomal formulation significantly affect drug release and anti-diabetic efficacy. Metformin niosomal formulations were characterized by controlled drug release along with effective plasma glucose levels reduction; thus, they may be regarded as an option for oral drug delivery system. According to our data, among all tested formulations, F2 showed the greatest therapeutic efficiency, whereas F3 had the best in vitro drug release characteristics. Hence, the proper selection and optimization of formulation composition are necessary in order to obtain a proper compromise between controlled

drug release and efficiency. Such data confirm the possibility of the utilization of the niosome-based drug delivery system in the delivery of metformin hydrochloride.

5.0. Conclusion and Further Studies

5.1. Conclusion

The current study is a presentation on the preparation of metformin hydrochloride-loaded niosomes using the method of ether injection and an assessment of their capability to serve as a controlled oral delivery system of the drug. In vitro release studies and the anti-hyperglycemic activity of the studied formulations revealed that the performance of the formulations depended significantly on the concentration of Tween 80. The best in vitro release rate of the drug was achieved by F3 (74.67%), while the maximum in vivo antihyperglycemic effect was obtained for F2 (63.15%) followed by F1.

In summary, it can be concluded that metformin hydrochloride-loaded niosomes have the capability to provide a controlled drug release and reduce the level of glucose in blood plasma. However, further research on the characterization of the vesicles, drug entrapment efficiency, pharmacokinetics, oral bioavailability, and stability is required to confirm the validity of the findings.

5.2. Further Studies

In order to further substantiate and elaborate on the results obtained from this study, the following research can be undertaken:

- a) Study the entrapment efficiency of the optimized niosomal formulation.
- b) Study the size, structure, zeta potential, and stability of the formulations.
- c) Study the oral bioavailability and plasma profile of the optimized formulation.
- d) In vivo study of the antidiabetic efficacy of the optimized formulation for prolonged periods of time.
- e) Perform stability studies on the formulation under different conditions.
- f) Compare the optimized formulation with conventional formulations of metformin.
- g) Perform clinical trials to prove the safety and efficacy of metformin-loaded niosomes in patients with type 2 diabetes mellitus.

Such researches will give a comprehensive information about pharmaceutical and therapeutic advantages of metformin hydrochloride-loaded niosomes as a controlled oral drug delivery system.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contributions

Md. Mahbubol Alam and Fahamida Binta Anower took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Kazi Nadia Haque Sharna, Tasin Ahmed Fahim, Md. Minhajul Alam, Eity Akter, and Tania Ferdous Urmi analyzed data. Tasin Ahmed Fahim, and Md. Minhajul Alam were involved in the design of the manuscript. All authors have read and approved the final manuscript.

Availability of Data and Materials

Supplementary information is available from the authors upon reasonable request.

Institutional Review Board Statement

Not applicable.

Ethical Approval

All experimental procedures involving albino mice were reviewed and approved by the Ethical Review Committee of Bangladesh University Research Center (BURC), Bangladesh (Approval No. BU/ERC/2025-01). Every effort was made to ensure the humane handling of animals and to minimize pain, discomfort, and stress throughout the experimental procedures.

Informed Consent

Not applicable for this study.

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Declaration of Artificial Intelligence

No artificial intelligence tools were used in this study.

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