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In-vitro and *In-vivo* Evaluation of Glimepiride Loaded Liposomes

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ABSTRACT

Liposomal drug delivery system of Glimepiride was made by ethanol-injection method. Soybean phospholipids and cholesterol with phosphate buffer saline were used to make liposomal suspension. Different characters of liposomes like drug encapsulation efficiency, turbidity and morphology were studied. Liposomes in size range of 99.5 nm to 320.6 nm were obtained. Encapsulation efficiency was found to from 79.6-97.5%. Turbidity studies showed high concentration surfactant formation. The SEM and TEM images showed liposomes are spherical shape. In-vitro drug release was studied using dynamic dialysis method. Biological studies were studied using albino rats. To determine the comparative leakage, stability studies were done at different temperatures.

Keywords: Antidiabetic liposomes, Glimepiride, Ethanol injection method, Albino rats

INTRODUCTION

Conventional antidiabetic drugs administered either orally or parenterally have several disadvantages. Liposomal drug delivery system is now becoming well established because of their ability to buffer the toxicity of entrapped drugs while maintaining efficacy [1]. The therapeutic areas of liposomes were widely extended as carriers for antidiabetics, anticancer agents, antibacterial, antifungal drugs and ocular liposomes. These studies show that the role of liposomes in satisfying pharmaceutical considerations is unavoidable in the field of medicine. The supporting factors of liposomes include inexpensive material, straightforward and rapid method of generating liposomes, homogeneous and reproducible size distribution and different efficient techniques for loading liposomes [2]. In addition, the final liposomal formulation must be highly stable, as both the retention of entrapped drug as well as chemical and dimensional stability of the liposome themselves.

For treating patients with type II diabetes mellitus, an oral antidiabetic drug, Glimepiride is commonly prescribed. Glimepiride is a sulfonylureas class drug with molecular formula $C_{24}H_{34}N_4O_5S$. The chemical structure of Glimepiride is displayed in the Figure 1. The weak acidic drug, Glimepiride, has its pKa value as 6.2. Glimepiride has no practical solubility in water and acidic environment

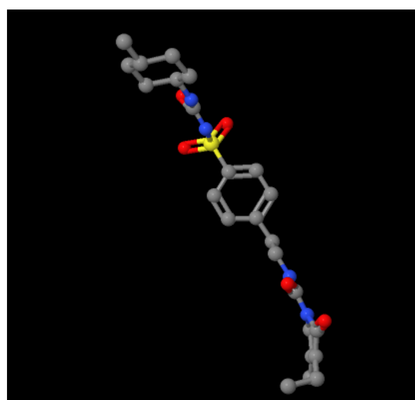

Figure 1: Structure of Glimepiride

but has high permeability (class II) [3]. The main aim of this study was to improve the solubility, dissolution property and controlled releasing property of the drug. Using modified ethanol injection method, liposomes were formulated. The liposomal formulations of Glimepiride were prepared by using soybean phospholipids and cholesterol.

MATERIALS AND METHODS

Materials

Glimepiride was a gift sample from Dr. Reddy's Laboratories, Hyderabad. Soybean phospholipids, cholesterol and potassium bromide were purchased from Sd fine chemical Mumbai. Streptozotozin was purchased from Himedia Pvt Ltd, Mumbai. All other chemicals and reagents used were of analytical grade.

Preparation of liposomes

Glimepiride loaded liposomes were prepared by modified ethanol injection method [4]. Required quantities of soybean phospholipid and cholesterol were weighed and transferred to ethanol solution. The solids were dissolved in ethanol. With continuous magnetic stirring the organic phase was slowly injected to the aqueous phase. 55 ± 2ºC temperature was maintained during the formulation [5]. Liposomes formed spontaneously as soon as ethanolic solution was in contact with aqueous phase. Phosphate buffer saline at pH 7.0 was used as aqueous phase. To remove the traces of solvent the Glimepiride loaded liposomes were kept at room temperature with constant stirring.

Drug entrapment studies

After rupture of the prepared Glimepiride loaded liposomes, the entrapment efficiency was determined. It was done using absolute alcohol for 10 min. Drug concentration was estimated by measuring the absorbance at 227 nm in triplicate using spectrophoto-meter with reference to the blank solution prepared (Table 1) [6].

Table 1: Encapsulation efficiency of Glimepiride loaded liposomes

CODE	Encapsulation Efficiency (%)
FA 1	82.42 ± 2.45
FA 2	92.62 ± 2.37
FA 3	97.55 ± 2.59
FA 4	89.71 ± 2.31
FA 5	88.54 ± 2.32
FA 6	79.60 ± 2.19
FA 7	89.25 ± 2.21
FA 8	85.35 ± 2.52
FA 9	89.77 ± 2.43

Microscopy

Every sample prepared was examined under optical microscope using oil immersion lens (Figure 2). Electron microscopic studies were carried out on transmission electron microscope (model JEM 1010®, Jeol (USA). After dilution of the Glimepiride loaded liposomal suspension with phosphate buffer it was stabilized with glutaraldehyde. Grids precoated with carbon film were used. It was examined under transmission electron microscope and photographs were taken and analysed using soft-imaging software (Figure 3). Scanning electron microscopic studies were carried out on scanning electron microscope (SEM) (model JSM-5610LV, Japan). It was operated at 15 KV with 80 mA. 240 micron diameter lens aperture was used (Figure 4) [7].

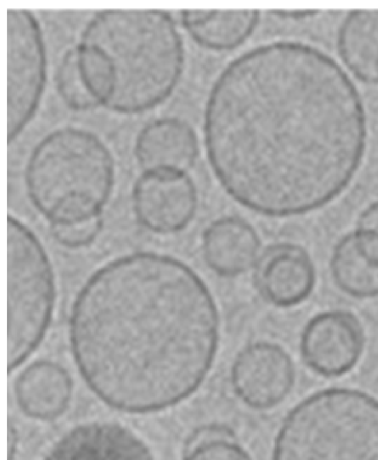

Figure 2: Optical microscopic image of Glimepiride formulations FA 3

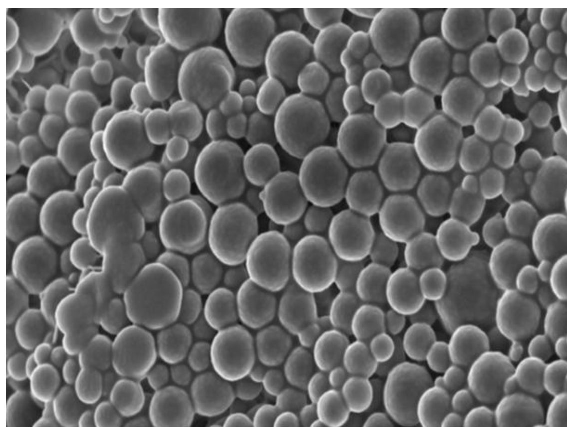

Figure 3: SEM image of formulation FA 3

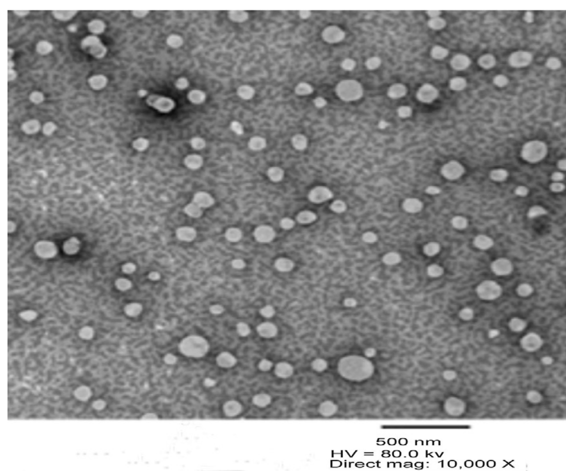

Figure 4: TEM image of formulation FA 3

Turbidity measurement

For 5 min the liposomes were rapidly mixed by sonication [8]. The turbidity was measured as the absorbance at 227 nm using UV-visible spectrophotometer (Table 2).

Table 2: Turbidity of Glimepiride loaded liposomes

CODE	Turbidity
FA 1	123 ± 2.3
FA 2	183 ± 2.2
FA 3	219 ± 3.2
FA 4	194 ± 2.4
FA 5	230 ± 5.2
FA 6	202 ± 3.2
FA 7	192 ± 4.2
FA 8	169 ± 2.5
FA 9	188 ± 3.9

***In-vitro* drug release**

In-vitro drug release was determined with the help of dialysis tubing (10,000 MWCO, Millipore, Boston, USA) using dynamic dialysis method [9]. Dialysis was performed until no drug content could be found in the solution. The dialysis bag was then immersed in 100 ml of phosphate buffer (pH 7.4). The temperature was maintained at 37 ± 0.5ºC and magnetically stirred at 50 rpm. At various time intervals 1 ml of sample was withdrawn and spectrophotometrically analysed. Results were tabulated and graph was plotted as percentage drug release versus time for formulations (Table 3 and Figure 5).

Biological studies

FA3 formulation of liposomes was selected for animal studies. (Registration number: 661/02/c/CPCSEA&19/07/2002. Proposal number: IEAC/KMCP/240/2015-2016) Studies were carried out in 24 albino rats divided into four groups with 6 animals each. First group as normal control, treated with water, second group as diabetic control treated with streptozotocin only, third group treated with streptozotocin and liposomal formulation FA3, whereas fourth group treated with streptozotocin and 5 mg/kg standard

Table 3: *In-vitro* drug release for FA3

Time	Cumulative % drug release of formulation FA3
1	25.42
2	30.09
4	43.93
8	61.74
12	78.85
16	83.96
20	90.07
22	95.17
24	99.28

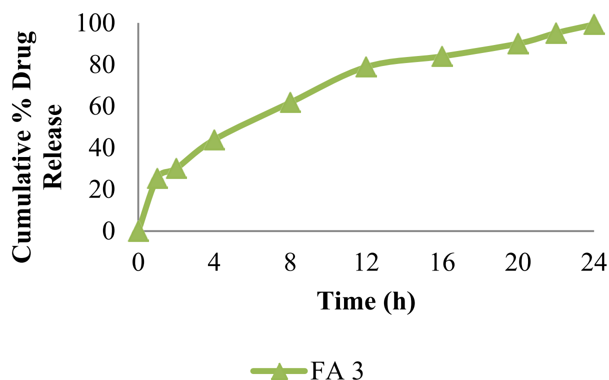

Figure 5: *In-vitro* drug release graph of FA 3

Glimepiride. The rats were confirmed to be diabetic by taking blood glucose levels above 200 mg/dl as a benchmark. ACCU-CHECK sensor (Roche Diagnostics) was used to check the Blood Glucose Level (BGL). The blood glucose levels were measured at different time intervals over six hours. Blood was obtained from the orbital sinus of animal (Tables 4 and 5) [10].

Table 4: Anti-diabetic activity blood glucose level profile

S. No.	Group	Blood Glucose Level (mg/dl) at different hours after Treatment								
		0 h	1 h	2 h	4 h	6 h	10 h	12 h	16 h	24 h
	Normal	86.5 ±5.3	85.6 ±1.5	83.2 ±7.8	82.3 ±6.2	84.4 ±4.1	83.2 ±6.5	87.5 ±2.2	85.8 ±5.9	80.6 ±6.2
	Diabetic Control	291.4 ±8.2	288.3 ±7.6	284.4 ±6.2	280.6 ± 9.3	283.5 ± 5.6	285.6 ± 9.5	277.4 ± 6.6	285.3 ± 4.8	284.3 ± 3.6
	FA 3	201.4 ± 2.1	281.3 ± 1.4	173.2 ± 2.3	156.4 ± 1.3	149.4 ± 1.2	110.2 ± 1.3	99.4 ± 2.9	98.2 ± 3.0	96.5 ± 2.1
	Standard Glimepiride	269.5 ± 5.8	259.2 ± 7.8	223.9 ± 6.5	143.7 ± 5.2	129.7 ± 8.9	162.6 ± 7.8	213.6 ± 4.3	231.2 ± 4.9	259.6 ± 8.7

Table 5: *In-vivo* kinetic parameters of FA3

Time (min)	Cp in µg/ml	Log Cp	$AUC_{(0-t)}$	Fa
0	0	-	-	-
60	0.900	-0.046	27.000	0.078
120	1.100	0.041	87.000	0.141
180	1.400	0.146	162.000	0.223
240	1.900	0.279	261.000	0.338
300	2.400	0.389	390.000	0.478
360	3.100	0.491	555.000	0.661
420	4.000	0.602	768.000	0.897
480	2.400	0.380	960.000	0.964
540	1.200	0.079	1068.000	0.983
600	0.700	-0.155	1125.000	1.001
660	0.300	-0.523	1155.000	1.002
720	0.100	-1.000	1167.000	1.000

Stability studies

The comparative leakage of the drug from liposomes stored at different conditions was done to find the physical stability of the Glimepiride loaded liposomes [11]. Different conditions like 4ºC ± 2ºC and 25ºC ± 2ºC, 60% RH ± 5% RH were used. Drug content of the liposomes were evaluated on 15th, 30th, 60th and 90th days (Table 6).

Table 6: Stability studies data of FA3

FA 3	Stored at 4°C (in days)				Stored at 25°C (in days)			
	15	30	60	90	15	30	60	90
Encapsulation Efficiency (%)	95.23 ± 3.2	92.31 ± 2.4	89.24 ± 1.2	86.23 ± 1.2	97.23 ± 3.2	97.31 ± 2.4	96.24 ± 1.2	95.23 ± 1.2

RESULTS AND DISCUSSION

The aim of the use of the drug loaded liposome in antidiabetic formulation is to sustain the release of the encapsulated drug. To achieve this goal, liposomes were prepared using various ratios of the components and evaluated. Modified ethanol injection method was used to prepare liposomes.

Drug entrapment efficiency

To find the best formulation entrapment efficiency was studied for all the 9 formulations. It was studied using the ratio between the total drug and the amount of drug encapsulated. The result shows that the encapsulation efficiency increased with increasing concentration of cholesterol.

Microscopy

Optical microscopy was found to be the fast and useful method for preliminary experiment. Transmission electron microscopy and Scanning electron microscopy enabled the determination of particle size of liposomes formed. The images prove the smooth nature and spherical shape of the liposomes, which confirms that the drug encapsulated did not affect the liposomes morphology (Figure 2-4).

Turbidity measurement

Turbidity measurement is an indication of solubility of drug in different concentration of tween 80 and cholesterol. The result shows that turbidity increases with increase in surfactant concentration. After optimum concentration it leads to formation of micelles.

In vitro drug release

The *in vitro* drug release of the formulations of Glimepiride loaded liposomes showed to have followed zero order kinetics. The Formulation FA3 showed the release of 99.28% at 24th hr.

Biological studies

The liposomal formulation showed a dose dependent blood glucose reduction in diabetic rats. The concentration of drug Glimepiride in plasma was used to calculate different parameters like C_{max}, T_{max}, $AUC_{0-t,}$ and Bioavailability by using Wagner-Nelson method. C_{max} (4 µg/ml) and T_{max} (420 min) were directly obtained from plasma concentration-time data.

Stability studies

The characteristics of liposomes were examined after 15, 30, 60 and 90 days. The results do not show any difference in physical characters of the liposomal formulations.

CONCLUSION

Glimepiride liposomes were successfully prepared by using modified ethanol injection method. Liposomes are somehow stable system for the targeted drug delivery. The size and size distribution analysis was carried out on selected formulations. The drug entrapment efficiency, morphology and turbidity of the optimized formulations were determined. *In-vitro* dissolution study was performed for all liposomal formulations. The maximum percentage of Glimepiride release was observed in the formulation FA3. From the research it was concluded that the Glimepiride is one of the good candidate for the successful development of liposomes for its therapeutic activity.

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