DEVELOPMENT OF AMORPHOUS LIPID-POLYMER HYBRID FOR IMPROVED SOLUBILITY OF A POORLY-WATER SOLUBLE-DRUG: GLIBENCLAMIDE AS A MODEL DRUG

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ABSTRACT

The use of glibenclamide in diabetes mellitus (DM) management is limited by poor aqueous solubility, dissolution rate, low bioavailability and short half-life. To address these problems, glibenclamide-loaded lipid-polymer hybrids (LPHs) were prepared. LPHs containing different ratios of beeswax and Eudragit® RS 100 as lipid and polymer, respectively, were prepared and characterized with respect to particle size, pH stability, encapsulation efficiency (EE %), loading capacity, in vitro release and in vivo bioactivity. The morphology and particle sizes showed smooth and spherical particles in the ranges of 14.22 ± 0.15 - 24.78 ± 0.31 µm, 8.84 ± 0.11 - 15.43 ± 0.16 µm, and 8.80 ± 0.11 - 11.00 ± 0.11 µm for X₁ – X₃, X₄ – X₆, and X₇ – X₉, respectively. The pH was stable without drug degradation. EE (%) of the LPHs ranged between 78.60 ± 0.02 - 95.40 ± 0.11 %, 91.33 ± 0.23 - 97.58 ± 0.17 %, and 89.66 ± 0.13 - 97.80 ± 0.18 % for X₁ – X₃, X₄ – X₆, and X₇ – X₉, respectively. In vitro release of the LPHs showed T₄₀ (time to release 40 % of the drug) at 4, 12, 0.5, 0, 12, 0.5, 0, 0, and 12 h for X₁ – X₉, respectively. Maximum blood glucose lowering (≈ 60 ± 22.6 mg/dl) was obtained in the LPHs, which was significant (p < 0.05) when compared to a commercial brand (69 ± 18.32 mg/dl). This study showed that LPHs is a promising alternative method for the delivery of a model poorly aqueous-soluble drug, glibenclamide.

KEYWORDS: Lipid-polymer hybrids; Antidiabetics; Hepatotoxicity; Glibenclamide; Blood glucose level; Beeswax.

INTRODUCTION

Diabetes mellitus is one of the most common metabolic diseases, globally. It is mainly classified as type I diabetes (insulin-dependent diabetes) and type II diabetes (non-insulin dependent diabetes). Hyperglycemia is a serious pathologic condition found in both types that can produce a variety of complications over a period of years, which include neurological and cardiovascular damages, and approximately 90 % of diabetic patients worldwide are affected by type II diabetes [1]. Glibenclamide is an antidiabetic drug that belongs to a sulfonylurea class with a remarkable therapeutic efficacy in the management of type II diabetes mellitus [2]. Sulfonylurea derivatives act as insulin secretagogues and trigger insulin release by inhibition of the ATP-sensitive potassium channels in the pancreatic islets [3, 4]. These derivatives increase insulin sensitivity from the β-cells of the pancreas [5].

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Glibenclamide belongs to class II drugs in the biopharmaceutical classification system due to its low poor water solubility (= 38 µmol/L) and good permeability properties [6]. This poor water solubility of the drug is responsible for its limited and variable oral bioavailability as well as problems of non-bioequivalence among its different commercial tablets [7]. Also, in the pharmacokinetic aspect, glibenclamide has a short T1/2 (half-life) within 8-10 h [8, 9]. Aqueous solubility influences the bioavailability of a given drug for efficient therapeutic response. Hence, drug solubility is a chief property that controls the drug’s major pharmacokinetic factors. Drug aqueous solubility has been a major challenge in formulation development owing to the fact that majority of newly discovered chemical compounds exhibit poor aqueous solubility. However, numerous techniques have been applied over the years aimed at improving the solubility and dissolution properties of poorly aqueous soluble drugs and modifying drug release to maintain plasma drug concentration and these include solid dispersions [10 – 12], cyclodextrins complexation [13], blends of polymers and cyclodextrins [14], liquid or solid self-microemulsifying drug delivery systems (SMEDDS) [18 – 20]. In particular, lipidic systems have been employed to enhance the solubility and bioavailability of glibenclamide [20, 21]. Although efforts have been made to improve the delivery of glibenclamide using lipids or polymers, none has exploited lipid-polymer hybrids. Therefore, in this study, we explored lipid-polymer hybrids (LPHs) as a delivery vehicle for glibenclamide. Lipid-polymer hybrid is an advanced smart lipid drug delivery that uses lipidic biomaterials and polymer blends [22]. The LPHs are lipid-based formulations that enhance aqueous solubility, limit biotoxicity of drugs, control and prolong drug release. Blends of biomaterials such as lipids and polymers have been reported to enhance sustained drug delivery of bioactives [24]. Lipids are important potential materials in drug formulation [25]. Lipids from plant origin have been found with limited harm [26]. The lipid part of LPHs could be solid wax such as beeswax with a long chain and high melting point [27]. Beeswax encapsulates active pharmaceutical ingredient (API) to prevent degradation and perhaps provide prolonged drug delivery. The ability of lipid-based matrices to sustain drug release of hydrophobic drugs has been demonstrated [28]. Some of the advantages of lipid-polymer technique over other colloidal drug delivery systems include improved drug stability, reduce biotoxicity, decreased enzymatic degradation, modulation of drug release pattern, high encapsulation efficiency, ease of scale-up, enhanced drug permeability due to lipid and stabilizers (surfactants) components, passive targeting, and improved bioavailability [29, 30]. More so, it has a good potential for achieving the aim of improving solubility, sustaining, targeting and controlling drug delivery.

Currently, there is paucity of information in the literature on the delivery of glibenclamide using lipid-polymer hybrids. Consequently, in this study, considering the challenges in the therapeutic application and efficacy of glibenclamide, including the low aqueous solubility, poor dissolution rate, low bioavailability, hypoglycemic adverse effect and short half-life [21, 22], the use of lipid-polymer based hybrid may be a suitable novel device for the delivery of glibenclamide to improve the solubility and poor dissolution challenges and sustain the drug release.

MATERIALS AND METHODS

Materials

Materials used in this study include glibenclamide powder (LKH3935, Wako Pure chemical industries, Ltd, China), Chitosan (KPK3395, MW 455,000 Da, Wako Pure chemical industries, Ltd, China), Poloxamer 407 (pluronic F-127), Eudragit® RS 100 (Evonik, Darmstadt, Germany). Wistar rats were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka. All chemicals used were analytical grades.

Preparation of glibenclamide-loaded lipid-polymer hybrids (LPHs)

The LPHs preparation using the emulsification-solvent evaporation (ESE) method as reported by previous researchers was adopted with little modification [31]. A 5 g quantity each of beeswax and Eudragit® RS 100 was weighed and dissolved in 10 ml of dichloromethane and stirred with a magnetic stirrer for 20 min to achieve a uniform solution. Then, 250 mg of glibenclamide (GLI) was incorporated and further homogenized using magnetic stirrer at 400 rpm for 30 min to obtain a lipid phase. An equivalent of 10 ml of aqueous preparations of chitosan (1 %), poloxamer 407 (2 %), and polyvinyl alcohol (PVA) (1%) each was added gently in a dropwise manner to the organic phase containing the glibenclamide and the dispersion was magnetically stirred for 20 min. The final mixture was subjected to sonication using the Ultrasonic Homogenizer (Probe Sonicator, Athena Technology, 80500W) 500W at 80 rev/sec for 60 seconds. The obtained LPHs after the evaporation of the solvent was collected and noted for 60 seconds. The obtained LPHs after the evaporation of the solvent was collected and noted for 60 seconds.
as formulations X₁, X₃, containing varying ratios of lipid and polymer, as shown in Table 1. The same procedure was repeated to load 500 mg and 1000 mg GLI and coded as formulations X₁ – X₆ and X₇ – X₁₀, respectively also containing varying ratios of lipid and polymer. The formulation compositions of the lipid-polymer hybrids are shown in Table 1.

Characterization of glibenclamide-loaded lipid-polymer hybrids (LPHs)

Morphology and particle size analysis
Approximately 2-3 drops of each formulation were viewed under a photomicroscope (Hund®, Weltzlar, Germany) attached with a digital camera at a magnification of 1000x and photomicrographs (Motacam, China) obtained. With the aid of the software in the photomicroscope, the particle morphologies were observed and photomicrographs taken. The sizes of the particles were measured (n = 30) and average taken.

Drug encapsulation efficiency (EE %) and loading capacity (LC)
Approximately 5 ml of the glibenclamide-loaded lipid-polymer hybrids were added into a microconcentrator (5000 MWCO Vivascience, Germany) and centrifuged (TDL-4 B. Bran Scientific and Instru. Co., London, England) at 3000 rpm for 2 – 3.5 h. The supernatants were decanted, filtered and appropriately assayed by UV/Vis spectrophotometer (Jenway 6405, Germany) at 230 nm previously obtained for glibenclamide. The quantity of drug encapsulated in the LPHs was calculated with reference to a standard Beer’s plot of GLI at predetermined wavelength of 230 nm. The experiment was carried out in triplicate and the average values ± S.D. were calculated. Then cumulative % drug released was obtained and plotted against each time interval. Then the release data obtained was analyzed using the Higuchi’s release kinetic model.

In vivo bioactivity study

Preparation of the experimental rats
Fifty male Wistar rats weighing between 185– 200 ± 10 g were procured and used for the experiment which complied with the ethics of animal use as per the National Code of Conduct for Animal Research Ethics (NCARE), with reference DOR/UNN/17/00012 obtained from our Institution Animal Ethical Committee which is in accordance with the requirements of the European Union Ethics on animal use [33]. Ab initio, the rats were supplied dry chick’s mash finisher for adult twice a day with free access to distilled water. They were housed separately in metabolic cages and allowed to acclimatize to the new experimental environment for two weeks, and consumption of food and water, urine volume and the levels of serum glucose were measured as a baseline before the induction of diabetes.

Induction of diabetes
The rats were fasted for 24 h prior to the induction of DM (diabetes mellitus). The baseline glucose determination was established. Fresh preparation of alloxan monohydrate solution (Sigma, USA) was used to induce diabetes. A stock solution of alloxan monohydrate was made by dissolving alloxan in normal saline (0.9% w/v NaCl) at a concentration of 100 mg/kg. An equivalence of 1 ml of the stock solution was given intraperitoneally after which the blood glucose levels were measured frequently for days using a glucometer (ACCU-CHEK Active, Roche Diagnostic GMBH, Germany). Diabetes was confirmed ≤ 5 days post-alloxan administration. Only
thirty-five rats were with an indication of diabetes signs and symptoms and were used for further studies.

**Oral administration of glibenclamide-loaded LPHs**

Thirty-five rats were randomly divided into seven groups of five animals per group for the investigation. Prior to the investigation, all rats fasted for twenty-four hours with access only to water. The first group (Placebo) received 2 ml blank formulation per oral (p.o). The second group received 2 ml of distilled water only p.o, while the third group received 5 mg pure glibenclamide dispersed in distilled water p.o and the commercial sample 5 mg was given to the fourth group. Then, the other groups of mice (fifth, sixth and seventh) also received orally 2 ml of selected formulations from each batch of the glibenclamide-loaded lipid-polymer hybrids; X5, X6, and X7, respectively. The selection of the formulation for the in vivo study was based on the formulation with the highest encapsulation efficiency.

**Toxicological Study**

Blood samples of five rats from each group (control, commercial sample, X3, X6, and X7) were withdrawn by puncturing the retro-orbital plexus under ether anesthesia. At room temperature, the withdrawn blood was allowed to clot for 30-40 min. Serum was separated by centrifugation at 2500 rpm for 15 min and various biochemical parameters were estimated and the livers of two animals were taken for the histopathology test. Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), and serum glutamic pyruvate transaminase (SGPT) were carried out using Lefotron machine and LFT kits.

**RESULTS**

**Morphology and particle size analysis**

The glibenclamide lipid-polymer hybrids were very smooth and spherical in shape though few deviated with irregular shape as presented in Figure 1. The particle size analysis of the formulations ranged between 14.22 ± 0.15 - 24.78 ± 0.31 µm, 8.84 ± 0.11 - 15.43 ± 0.16 µm, and 8.80 ± 0.11 - 11.00 ± 0.11 µm for X1 – X3, X4 – X6, and X7 – X9, respectively as shown in Table 2. The highest particle size for the glibenclamide-loaded LPHs was 24.78 µm (formulation X1), whereas the lowest particle size was 8.80 µm (formulation X9).

**pH stability studies**

The result of pH stability test of the LPHs carried out between 1 to 30 days as presented in Table 3 showed pH range of 4.2 ± 0.01 - 4.3 ± 0.00, 4.3 ± 0.00 - 4.3 ± 0.01, 4.0 ± 0.15 - 4.1 ± 0.17, 4.5 ± 0.12 - 4.6 ± 0.10, 4.5 ± 0.01 - 4.5 ± 0.05, 4.6 ± 0.02 - 4.7 ± 0.00, 4.4 ± 0.15 - 4.4 ± 0.01, 4.3 ± 0.00 - 4.3 ± 0.12 and 4.2 ± 0.01 - 4.2 ± 0.10 for LPH formulations X1 – X9, respectively. The pH of the LPH formulations was observed to be slightly acidic which may be as a result of fatty acids composition of the lipid. The stability studies of the LPHs showed an insignificant change (p > 0.05) in the pH over a period of 30 days, implying that there was no degradation of the drug and the excipients of the formulation within the period of study. Also, the amount of drug loaded in the formulation did not affect the stability of the LPH formulations as shown in Figure 2.

**Encapsulation efficiency (EE %) and loading capacity (LC)**

The encapsulation efficiency of the LPH formulations as shown in Table 2 ranged between 78.60 ± 0.02 - 95.40 ± 0.11 %, 91.33 ± 0.23 - 97.58 ± 0.17 %, and 89.66 ± 0.13 - 97.80 ± 0.18 % for X1 – X3, X4 – X6, and X7 – X9, respectively. The LPH formulations (X3, X6 and X9) with the highest lipid: polymer ratio had the highest EE (%) in each batch. The loading capacity increased with an increase in drug loading as shown in Table 2.

**In vitro drug release and release mechanism**

The in vitro release of GLI-loaded LPHs as presented in Figures 3 - 5 showed T50 (time to release 40 % of the drug) at 4, 12, 0.5, 0, 12, 0.5, 0, 0, and 12 h for formulations X1 – X9, respectively. Slight initial burst effects were observed in LPH formulations X1, X3, and X6 before a steadily maintained controlled drug release over extended period. This effect may be due to un-encapsulated drug that remained at the shell after the homogenization process. Higher concentration of polymer in some LPH formulations might also be attributed to the partial drug expulsion during formulation. The highest drug release of 98 % was observed in LPH formulation X1 (Figure 3) with equal lipid: polymer ratio (1:1), while the lowest drug release of ≈ 21 % was depicted in LPH formulations X4, X7 and X9 (Figures 4 and 5).
Table 1: Formulation composition of glibenclamide-loaded lipid-polymer hybrids

<table>
<thead>
<tr>
<th>Batch</th>
<th>Drug (g)</th>
<th>L:P ratio</th>
<th>PVA (ml)</th>
<th>PL (ml)</th>
<th>CH (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁</td>
<td>0.25</td>
<td>1:1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₂</td>
<td>0.25</td>
<td>3:1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₃</td>
<td>0.25</td>
<td>1:3</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₄</td>
<td>0.50</td>
<td>1:1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₅</td>
<td>0.50</td>
<td>3:1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₆</td>
<td>0.50</td>
<td>1:3</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₇</td>
<td>1.00</td>
<td>1:1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₈</td>
<td>1.00</td>
<td>3:1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X₉</td>
<td>1.00</td>
<td>1:3</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Key: L: P (lipid: polymer ratio); Polyvinyl alcohol (PVA); Poloxamer (PL); Chitosan (CH); X₁ – X₃, X₄-X₆ and X₇-X₉ contain increasing concentrations (0.25, 0.5 and 1.0 g) of glibenclamide, respectively; X₁, X₄ and X₇ contain beeswax and Eudragit at 1:1 ratio; X₂, X₅ and X₈ contain beeswax and Eudragit at 3:1 ratio, whereas X₃, X₆ and X₉ contain beeswax and Eudragit at 1:3 ratio.

Figure 1: Photomicrograph of glibenclamide-loaded LPHs (X₁ –X₉), respectively.

Key: X₁ – X₉ (LPH formulations); X₁ – X₃, X₄-X₆ and X₇-X₉ contain increasing concentrations (0.25, 0.5 and 1.0 g) of glibenclamide, respectively; X₁, X₄ and X₇ contain beeswax and Eudragit at 1:1 ratio; X₂, X₅ and X₈ contain beeswax and Eudragit at 3:1 ratio, whereas X₃, X₆ and X₉ contain beeswax and Eudragit at 1:3 ratio.
Table 2: Encapsulation efficiency and drug loading capacity and Particle size of glibenclamide LPHs

<table>
<thead>
<tr>
<th>Batch</th>
<th>EE (% ± SD)</th>
<th>LC (% ± SD)</th>
<th>P.S (µm ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁</td>
<td>78.60 ± 0.02</td>
<td>12.50 ± 0.22</td>
<td>24.78 ± 0.31</td>
</tr>
<tr>
<td>X₂</td>
<td>88.86 ± 0.12</td>
<td>6.25 ± 0.11</td>
<td>14.22 ± 0.15</td>
</tr>
<tr>
<td>X₃</td>
<td>95.40 ± 0.11</td>
<td>6.25 ± 0.31</td>
<td>18.74 ± 0.11</td>
</tr>
<tr>
<td>X₄</td>
<td>91.33 ± 0.23</td>
<td>25.00 ± 0.18</td>
<td>15.43 ± 0.16</td>
</tr>
<tr>
<td>X₅</td>
<td>92.70 ± 0.03</td>
<td>12.50 ± 0.11</td>
<td>8.84 ± 0.11</td>
</tr>
<tr>
<td>X₆</td>
<td>97.58 ± 0.17</td>
<td>12.50 ± 0.12</td>
<td>10.47 ± 0.21</td>
</tr>
<tr>
<td>X₇</td>
<td>89.66 ± 0.13</td>
<td>50.00 ± 0.05</td>
<td>8.80 ± 0.11</td>
</tr>
<tr>
<td>X₈</td>
<td>91.46 ± 0.21</td>
<td>25.00 ± 0.11</td>
<td>11.00 ± 0.11</td>
</tr>
<tr>
<td>X₉</td>
<td>97.80 ± 0.18</td>
<td>25.00 ± 0.06</td>
<td>9.90 ± 0.14</td>
</tr>
</tbody>
</table>

Key: X₁ – X₉ (LPH formulations); X₁ – X₃, X₄-X₆ and X₇-X₉ contain increasing concentrations (0.25, 0.5 and 1.0 g) of glibenclamide, respectively; X₁, X₄ and X₇ contain beeswax and Eudragit at 1:1 ratio; X₂, X₅ and X₆ contain beeswax and Eudragit at 3:1 ratio, whereas X₃, X₆ and X₉ contain beeswax and Eudragit at 1:3 ratio; EE (encapsulation efficiency); LC (loading capacity), P.S (particle sizes); SD (Standard deviation).

Table 3: The pH of the LPH formulations

<table>
<thead>
<tr>
<th>Batch</th>
<th>Day 7 (AV ± SD)</th>
<th>Day 14 (AV ± SD)</th>
<th>Day 30 (AV ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁</td>
<td>4.2 ± 0.01</td>
<td>4.2 ± 0.02</td>
<td>4.3 ± 0.00</td>
</tr>
<tr>
<td>X₂</td>
<td>4.3 ± 0.00</td>
<td>4.3 ± 0.00</td>
<td>4.3 ± 0.01</td>
</tr>
<tr>
<td>X₃</td>
<td>4.0 ± 0.15</td>
<td>4.1 ± 0.15</td>
<td>4.1 ± 0.17</td>
</tr>
<tr>
<td>X₄</td>
<td>4.5 ± 0.12</td>
<td>4.6 ± 0.12</td>
<td>4.6 ± 0.10</td>
</tr>
<tr>
<td>X₅</td>
<td>4.5 ± 0.01</td>
<td>4.5 ± 0.01</td>
<td>4.5 ± 0.05</td>
</tr>
<tr>
<td>X₆</td>
<td>4.6 ± 0.02</td>
<td>4.7 ± 0.02</td>
<td>4.7 ± 0.00</td>
</tr>
<tr>
<td>X₇</td>
<td>4.4 ± 0.15</td>
<td>4.4 ± 0.15</td>
<td>4.4 ± 0.01</td>
</tr>
<tr>
<td>X₈</td>
<td>4.3 ± 0.00</td>
<td>4.2 ± 0.00</td>
<td>4.3 ± 0.12</td>
</tr>
<tr>
<td>X₉</td>
<td>4.2 ± 0.01</td>
<td>4.2 ± 0.05</td>
<td>4.2 ± 0.10</td>
</tr>
</tbody>
</table>

Key: X₁ – X₉ (LPH formulations); X₁ – X₃, X₄-X₆ and X₇-X₉ contain increasing concentrations (0.25, 0.5 and 1.0 g) of glibenclamide, respectively; X₁, X₄ and X₇ contain beeswax and Eudragit at 1:1 ratio; X₂, X₅ and X₆ contain beeswax and Eudragit at 3:1 ratio, whereas X₃, X₆ and X₉ contain beeswax and Eudragit at 1:3 ratio; AV (average mean); SD (Standard deviation).

Figure 2: Glibenclamide-loaded LPHs after the stability study.
Figure 3: Percentage glibenclamide released of X₁ – X₃ formulations.
Key: X₁, X₂ and X₃ containing 1:1, 3:1 and 1:3 of beeswax and Eudragit RS100 loaded 250 mg of glibenclamide each.

Figure 4: Percentage drug released of X₄ – X₆ formulations
Key: X₄, X₅ and X₆ containing 1:1, 3:1 and 1:3 of beeswax and Eudragit RS100 loaded with 500 mg of glibenclamide each.

Figure 5: Percentage drug released of X₇ – X₉ formulations
Key: X₇, X₈ and X₉ containing 1:1, 3:1 and 1:3 of beeswax and Eudragit RS100 loaded with 1000 mg of glibenclamide each.
Table 4: Mechanism of released of glibenclamide from the formulations

<table>
<thead>
<tr>
<th>Batch</th>
<th>$K_H$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
<td>1.115</td>
<td>0.959</td>
</tr>
<tr>
<td>$X_6$</td>
<td>0.8390</td>
<td>0.627</td>
</tr>
<tr>
<td>$X_9$</td>
<td>0.4590</td>
<td>0.965</td>
</tr>
<tr>
<td>Commercial sample</td>
<td>0.1593</td>
<td>0.959</td>
</tr>
</tbody>
</table>

Key: $K_H$ = Release kinetics constant; $R^2$ = Regression coefficient

Figure 6: Effect of glibenclamide-loaded LPHs on blood glucose levels.
Key: $X_3$, $X_6$ and $X_9$ are LPH formulations containing beeswax and Eudragit at 1:3 ratio; $X_3$, $X_6$ and $X_9$ contain increasing concentrations (0.25, 0.5 and 1.0 g) of glibenclamide, respectively.

Figure 7: Effect of the GLI-loaded LPHs on the Liver enzymes.
Key: $X_3$, $X_6$ and $X_9$ are LPH formulations containing beeswax and Eudragit at 1:3 ratio; $X_3$, $X_6$ and $X_9$ contain increasing concentrations (0.25, 0.5 and 1.0 g) of glibenclamide, respectively.
In vivo antidiabetic studies of glibenclamide-loaded LPHs on the experimental rats

High intake values of 150 ± 5 ml (water) and 50.6 ± 4 g (food) were observed in an alloxan made diabetic rats, as compared to the values of 35 ± 5 ml (water) and 11.3 ± 3 g (food) observed in healthy adult rats. It was observed that daily urine of 11.1 ± 5 ml (healthy rats) and 130 ± 5 ml (diabetic rats). The in vivo antidiabetic study as shown in Figure 6 indicated that the group that received blank sample (placebo) and water continued to have elevated blood glucose levels (≈ 129 mg/dl) throughout the 24 h experimental period with some animals dying. However, the group that received pure sample of GLI achieved 94 ± 15.6 mg/dl glucose-lowering at the end of the experiment. The glibenclamide-loaded LPH formulations X₃, X₄, and X₅ lowered the blood glucose levels of diabetic rats. The initial loading dose (burst) of LPH formulations X₁ and X₆ was remarkably depicted with blood glucose-lowering (≈ 80 mg/dl) within 0.5 h of drug administration. Maximum blood-glucose-lowering (≈ 60 ± 22.6 mg/dl) was observed in the LPH formulations when compared to the blood glucose reduction (69 ± 18.32 mg/dl) of the conventional GLI tablet sample which only released within 8 h, while the LPH prolonged the drug release for 10 h.

Toxicological study

The result of the liver function tests (LFT) as presented in Figure 7 indicated that the SGPT, SGOT and ALP levels were 39.6, 70.5 and 115.0 IU/L for control, 38.5, 72.5 and 119.2 IU/L for commercial sample, 39.1, 66.5 and 115.0 IU/L for formulation X₃, 38.5, 68.5 and 119.2 IU/L for formulation X₅ and 34.5, 68.2 and 120.2 IU/L for formulation X₆ respectively.

DISCUSSION

The smaller particle sizes were observed in the LPH formulations with higher lipid component (3:1) which is an indication that the drug solubilized more with reduced particle sizes in the lipid components. The variation in particle sizes may be due to the differences in the lipid:polymer ratios. The hybrid formulations with equal lipid:polymer ratios (1:1) exhibited the highest particle sizes, unlike other ratios that gave lower particle sizes.

This physical stability effect observed might be the effects of the incorporated stabilizers (Poloxamer 407 and PVA) buttressed with poly-dispersed reduced particle sizes of the LPH formulations. The amount of drug loaded in the formulation did not affect the stability of the LPH formulations which is in agreement that lipid-polymer provides stability which inhibits the degradation of the encapsulated drug [34]. In addition, solid lipid (beeswax) has established potential of controlling drug release and inhibiting chemical degradation of encapsulated drug [35].

The LPHs were able to accommodate a high amount of the drug since the EE (%) was ≥ 79 %, which is an important quality of LPH (solid lipid matrices) formulations. The high EE (%) and LC may be as a result of the hybridization of lipid and polymer matrix, presence of surfactants, and beeswax containing different chain length resulted into the less perfect crystal to provide more spaces to accommodate the drug [23, 24]. Also, an earlier report showed that nature of lipidic biomaterials, preparation technique and lipophilic nature of the drug could determine the EE (%) and LC [32].

In the management of diabetes, burst effects are desirable since hyperglycemia is an emergency situation which requires immediate blood sugar reduction. Thus, the initial drug release may serve as loading dose and the gradual prolonged drug release may then serve as maintenance dose, consistent with earlier reports [36 – 39]. This low or prolonged delayed drug release observed in some LPH formulations was as a result of good drug encapsulation in the matrices which controlled such that they could not release up to 40 % of their drug content within 12 h. The prolonged drug release is important since gradual drug release will uniformly occur from the entrapped drug in the LPH matrices as a result of slow rate of hydration and gelling exhibited by the excipients employed. Drugs which were embedded in beeswax matrices have been reported to exhibit retarded drug release, which is in line with the beeswax-based formulations obtained in this study [32, 40, 41]. Lipid formulation (solid lipid matrix) would equally avoid rapid drug release and in another way controls drug mobility [42]. This showed that glibenclamide-loaded LPH formulations gave improved drug release to maintain the required blood sugar level. In addition, as a result of improved diffusional pathway, the drug release should be sustained [43, 44]. In the release mechanism of glibenclamide, Higuchi model describes release of drug from an insoluble matrix as square root of time and depicted R² value of 0.959, 0.627, 0.965 and 0.959 for LPH formulations X₁, X₄, and X₅, respectively (Table 4). This depicted that all except formulation X₁ followed this model with drug release diffusion mechanism. This is consistent with a report that gradual phase of drug release from lipid matrix is attributed to diffusion-controlled effects [45].
presence of diabetic condition. These features could be as a result of the fast changes in the physiologically condition of the animal due to insulin deficiency. There was break down of the fat tissue in the rat leading to loss of weight as observed in the diabetic rats and consequently the thinness of diabetic rats. The blood glucose levels of the rats were increased and remained high three-day post-alloxan administration. In addition to the maintained hyperglycemia, the rats showed polyurea, polydipsia, polyphagia, and weight loss as stated earlier. The animals with the blood glucose level up to 100 mg/dl were considered diabetic [46] especially as the animals fasted for 12 h with access to water only.

The in vivo study result was an indication that the release of glibenclamide from the LPHs stimulated the production of insulin from islet cells of Langerhans in a much more controlled manner over a period of 10 h by the prolonged release of glibenclamide from the LPHs than the conventional tablet form. Hence, glibenclamide-loaded LPHs enhanced the therapeutic activity of the drug which could be effectively delivered as LPHs. The toxicological results obtained showed that the values of these enzyme markers were within the normal range since the standard range value include 30 – 130 IU/L for ALP, 50 – 150 IU/L for SGOT and (10 – 40 IU/L) for SGPT [47, 48]. Therefore, delivery of glibenclamide as LPH has no toxicity effect in the liver enzymes.

CONCLUSIONS

Lipid-polymer hybrid (LPHs) is an innovative technology that is used to improve the delivery of poorly-soluble drugs like glibenclamide. The encapsulation efficiency of glibenclamide-loaded LPH formulations provided impressive results. The dissolution rate of glibenclamide was improved by preparing drug-loaded LPHs using the emulsification solvent evaporation (ESE) method. The in vivo study shown that glibenclamide-loaded LPHs could deliver and sustain the release of glibenclamide in diabetic rats than the conventional drug. It follows that LPHs could be an alternative to the tablet dosage form for the control of hyperglycemia in diabetic patients.

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