SMEDDS FORMULATION: DEMONSTRATION OF ENHANCED BIOAVAILABILITY OF PIOGlitAZONE IN RATS

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ABSTRACT
Objective: The present study was aimed at kinetic evaluation of Pioglitazone (Pio) in a novel self micro-emulsifying drug delivery system (SMEDDS) for enhanced oral administration. Poor water solubility of the drug has envisaged this work to prove with kinetic data the enhanced solubility by using novel SMEDDS systems.

Methods: SMEDDS of Pio consisted of cotton seed oil as oily phase, tween 80 as the surfactant and PEG 400 as the co-surfactant containing 15 mg/ml of Pio. The optimized formulation selected by the aid of pseudo-ternary phase diagrams; confirmed increase in drug release by in vitro dissolution studies, with enhanced drug diffusion through biomembranes demonstrated by ex vivo intestinal diffusion studies, with SEM and TEM studies of reconstituted SMEDDS as a proof of nano size range and spherical shape of the droplets was selected for the kinetic study in rats.

Pio SMEDDS formulation was administered orally to one study group and plain drug suspension to the other group. The plasma drug concentration was estimated by validated RP-HPLC method after successful plasma spiking of the drug.

Results: An increase in Cmax and AUC values (p< 0.05) for the SMEDDS formulation depicts the increase in bioavailability of otherwise poorly soluble drug in comparison with plain drug formulation. A 3 month stability studies at accelerated conditions (40°C & 75% RH) showed no change in physical appearance, droplet size and dissolution rate of the drug.

Conclusion: Thus SMEDDS formulation was found to be instrumental in improving oral bioavailability and thus therapeutic efficacy of Pioglitazone.

Keywords: Tween 80, Phase diagrams, TEM, Plasma spiking, Biomembranes.

INTRODUCTION
Pioglitazone is a thiazolidine derivative which decreases insulin resistance by its action at PPAR-γ receptors.[1,2] Its very poor water solubility has limited its efficacy and bioavailability. Hence a novel self microemulsifying drug delivery system (SMEDDS)[3] was improved and evaluated for enhanced bioavailability of pioglitazone. SMEDDS systems comprises of oils, surfactants and co-surfactants as excipients intimately mixed to form a fine oil in water emulsion upon dilution with GI fluids to achieve nanosized drug droplets for excellent solubility.[4,5]

Many studies were conducted on pioglitazone as formulations in solid dispersions[6,7], inclusion complexes [8,9] and fast dissolving formulations [10]. However this study emphasizes on SMEDDS formulation of pioglitazone and evaluation of kinetic parameters by conducting bioavailability studies in rats [11,12]. Wherein the otherwise poorly water soluble drug was demonstrated to show increase in bioavailability as confirmed by the kinetic data obtained from the study.

MATERIALS AND METHODS

Formulation design of SMEDDS containing pioglitazone
According to solubility analysis and phase diagrams studies; the formulations were prepared by initially dissolving pioglitazone in PG/400 (cosurfactant) at 60° C in an isothermal water bath, cottonseed oil was then added and mixture was cooled to ambient temperature, then tween 80 (surfactant) as shown in table 1, was added and the final mixture was sonicated to get a clear solution. The formulation was equilibrated at ambient temperature for at least 48 hours and examined for signs of turbidity (or) phase separation [13].

The liquid SMEDDS were solidified by adsorbing them onto microcrystalline cellulose which serves as carrier. In all the formulations, the drug concentration was constant as 15 mg/ml of liquid SMEDDS.

Evaluation of Pio SMEDDS
The prepared formulations were evaluated for physical appearance, dynamic light scattering was used to characterize nanostructuring, TEM[14] and SEM studies[15] were done for surface characteristics, dissolution studies [16] and Ex-vivo intestinal studies were done to estimate drug release and diffusion [17].

In vivo studies

HPLC analysis of Pio in rat plasma:
The concentration of Pio in rat plasma was determined by validated HPLC method using C18 column, with water and acetonitrile as mobile phase (50:50) at 1ml/min flow rate, detected at 225nm of uv detection. 200µl of plasma containing drug was mixed with 200µl of acetonitrile, vortexed for 5 min, centrifuged at 5000 rpm for 15 min. the supernatant of 20µl was injected into HPLC [18,19]

Animals

Healthy male wistar rats weighing 300-350g were selected and housed with institutional guidelines, fasted overnight and had free access to drinking water. All the experiments were performed after receiving approval of the institutional animal care committee of KLE university Belgaum [20].

Experimental design

Animals were separated into two experimental groups, the first group consisted of rats given SMEDDS pio and the second group contained rats given a suspension of plain pio (0.25% CMC Na). The drug was administered orally without sedation into the stomach at a dose of 10 mg/kg body weight.

Blood samples (pp 0.5ml) were collected from retro orbital plexus pre dose for plasma drug spiking and the 15min, 30min, 1,2,4,6,8,12 & 24hrs after drug administration into heparinised tubes. The blood was centrifuged at 5000rpm in cooling centrifuge and plasma was separated, stored at -20°C until further use [21].

Data analysis

The pharmacokinetic parameters were calculated by using one compartment model and peak time (t_{max}), peak level Cmax were estimated by PK solver. Area under the whole blood concentration
time curve (AUCtot) was also calculated by the trapezoidal rule for the mean whole blood levels.

All results were expressed as mean ±SD. Differences between two related parameters were assessed by student t test or one way anova by using graph pad prism software [22,23].

Stability studies

Chemical and physical stability of solid and liquid Pio SMEDDS were assessed at storage conditions of 40 ±2°C/75 %RH. The samples were evaluated at 1,2,3 months for physical appearance and drug content and particle size was estimated at end of three months [24,25].

RESULTS AND DISCUSSION

Formulation design of SMEDDS containing pioglitazone:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Tween 80</td>
<td>5.1</td>
<td>5.0</td>
<td>5.1</td>
<td>5.4</td>
<td>5.5</td>
<td>5.5</td>
<td>5.2</td>
<td>5.4</td>
<td>5.6</td>
<td>5.6</td>
<td>5.4</td>
<td>5.5</td>
<td>6.2</td>
<td>6.1</td>
<td>6.2</td>
</tr>
<tr>
<td>% PEG-400</td>
<td>27.0</td>
<td>30.4</td>
<td>25.2</td>
<td>22.0</td>
<td>23.9</td>
<td>17.6</td>
<td>17.2</td>
<td>32.5</td>
<td>12.3</td>
<td>11.3</td>
<td>16.1</td>
<td>6.2</td>
<td>30</td>
<td>36.1</td>
<td>48.2</td>
</tr>
<tr>
<td>% cottonseed oil</td>
<td>18</td>
<td>18.6</td>
<td>23</td>
<td>22.4</td>
<td>10.5</td>
<td>27.1</td>
<td>28.3</td>
<td>31.5</td>
<td>31.4</td>
<td>33.9</td>
<td>16.1</td>
<td>6.2</td>
<td>30</td>
<td>36.1</td>
<td>48.2</td>
</tr>
<tr>
<td>% Glycerol</td>
<td>27.9</td>
<td>31.2</td>
<td>8.6</td>
<td>21.2</td>
<td>16.8</td>
<td>12.3</td>
<td>11.3</td>
<td>6.2</td>
<td>30</td>
<td>36.1</td>
<td>48.2</td>
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</table>

Evaluation of Pio SMEDDS

The formulations when evaluated for phase separation and visibility grade were found to be stable with no phase separation and had visibility grade A. The emulsification time was found to be below 108 sec, with a cloud point of 80°C. Upon dilution with 6.8 phosphate buffer and distilled water there was no observed precipitation or cloudiness indicating the stability of SMEDDS on dilution. The targeted particle size was achieved within nanosized range as shown in table 2 & fig 1, with zeta potential being negative and PDI within the range. The optimized formulation when subjected to in vitro dissolution study on comparison to marketed formulation showed faster dissolution rate within 60 min as shown in fig 2, the SEM photograph of solid SMEDDS was clear evidence that the liquid SMEDDS was completely adsorbed onto the solid carrier which shows clear particles with roughened surfaces as shown in fig 3, the ex-vivo rat intestinal diffusion study has clearly depicted that the diffusion of the drug has been increased on comparison with plain drug formulation as seen in fig 4, the TEM photograph of optimised formulation seen in fig 5 is a clear evidence of decreased particle size as round particles in nanosize range can be noticed in the figure.

Table 2: Droplet size, zeta potential and polydispersity index of optimized SMEDDS of pioglitazone on dilution with water

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Particle Size(nm)</th>
<th>Zeta Potential(V)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4</td>
<td>10.75</td>
<td>-5.07</td>
<td>0.150</td>
</tr>
<tr>
<td>F5</td>
<td>90.26</td>
<td>-2.38</td>
<td>0.236</td>
</tr>
</tbody>
</table>
Stability studies of Pio SMEDDS: The stability studies conducted on optimized SMEDDS formulation has clarified that the liquid SMEDDS were stable for 3 months, with no physical changes and drug content when evaluated at the end of each month. The particle size of the formulation has shown no much change, with PDI and zeta potential too not much varied which is an authentication of good stability of the prepared formulations.

Table 4: Stability studies of Pio SMEDDS

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Physical appearance</th>
<th>Drug content (%)</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clear liquid</td>
<td>100.0±2.76</td>
<td>90.26</td>
<td>0.2</td>
<td>-2.38</td>
</tr>
<tr>
<td>1</td>
<td>Clear liquid</td>
<td>99.98±0.25</td>
<td>36</td>
<td>0.2</td>
<td>-2.28</td>
</tr>
<tr>
<td>2</td>
<td>Clear liquid</td>
<td>99.91±0.36</td>
<td>45</td>
<td>0.2</td>
<td>-2.43</td>
</tr>
<tr>
<td>3</td>
<td>Clear liquid</td>
<td>99.92±0.27</td>
<td>95.6</td>
<td>0.2</td>
<td>-2.43</td>
</tr>
</tbody>
</table>

In conclusion, the current study may evolve into an effective therapeutic technology for the treatment of diabetes mellitus.

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REFERENCES


