Research Article

Chronomodulated Nifedipine Supports Concurrent Glimepiride Administration with Subsequent Amelioration of Retinopathy and Peripheral Neuropathy in Diabetic Rats

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Abstract. This study investigated effect of chronomodulated nifedipine on two microvascular complications in glimepiride-treated diabetic rats for 21 days. Groups 1 and 2 were non-diabetic and diabetic controls respectively, receiving 1 ml/kg PEG+H₂O. Groups 3-5 were diabetic, receiving 10 mg/kg glimepiride at 2000 hrs. In addition, groups 4 and 5 received nifedipine, 20 mg/kg at 2000 hrs and 0800 hrs respectively. Peripheral neuropathy was evaluated weekly using the paw pressure and tail immersion tests, while retinopathy was evaluated by determining levels of some serum ocular markers, and histological assessment of retina. Treatment with glimepiride alone at 2000 hrs, produced no significant effect on the complications. Treatment with glimepiride and nifedipine at 2000 hrs exacerbated the complications. Conversely, treatment with glimepiride at 2000 hrs and nifedipine at 0800 hrs significantly (P<0.05) ameliorated both complications. These findings suggest that with chronotherapy, both drugs may be used together for the purpose of ameliorating retinopathy and peripheral neuropathy.

Keywords: Nifedipine, glimepiride, chronotherapy, retinopathy, peripheral neuropathy

1. Introduction

Circadian rhythm refers to endogenous activities that occur within a living organism in approximately twenty-four hours [1] and has long been established in man as it affects endogenous activities [2] and disease conditions [3]. It has also been reported that the time of drug administration, especially with reference to circadian rhythms, affects their pharmacokinetics and pharmacodynamics, hence variation in the therapeutic outcome of different classes of medications [4]. It is therefore important that drug treatment, especially in disease conditions like diabetes that exhibit rhythmicity [5], be timed to match the rhythm of disease.

Diabetes mellitus poses a serious public health problem, especially with increasing prevalence of type 2 diabetes mellitus worldwide [6]. Prolonged diabetes predisposes to microvascular complications such as retinopathy, peripheral neuropathy, and nephropathy which are leading causes of morbidity and mortality in diabetic populations worldwide [7]. These complications arise chiefly from persistent hyperglycaemia and uncontrolled diabetes; the major predisposing factors leading to the onset and progression of diabetic
microvascular complications [8]. While retinopathy is one of the leading causes of blindness worldwide [7], peripheral neuropathy poses the risk of diabetic foot ulcers, amputation and charcot joints [9]. Treatment thus requires the use of drugs that will control blood glucose to desired levels, coupled with measures to delay the progression and improve prognosis of microvascular complications.

Glimepiride, as well as other sulphonylureas have been widely prescribed especially for the management of type 2 diabetes mellitus [10]. Nifedipine, a calcium channel blocker is an effective nephro-protective agent [11] and has also shown promising potentials in the amelioration of peripheral neuropathy [12] and retinopathy [13] in diabetics. As a result, a regimen that contains both drugs will expectedly offer advantage against microvascular complications in diabetic patients. However, interaction between these drugs produces an unwanted effect [14] which could lead to loss of therapeutic benefits of either or both drugs. A previous study established that an alternate time of nifedipine administration through chronomodulation offers an acceptable and effective option to concurrent administration and offered reno-protection [15]. The observed reno-protection was as a result of chronotherapeutic considerations in nifedipine’s administration whose vasodilatory effect combined synergistically with endogenous processes involved in effective renal function and also as a result of diurnal variation in its pharmacokinetics. In the present study, we investigated the effect of 21-day chronomodulated nifedipine administration on glycaemic control and the prognosis of retinopathy and peripheral neuropathy in glimepiride-treated hyperglycaemic rats.

2. Materials and Method

2.1. Materials

2.1.1. Animals. This study was conducted according to the ethical guidelines on laboratory animal use and care policy, which is in compliance with the Ahmadu Bello University Research Policy. A total of forty male albino Wistar rats with a weight range of 130-150 g were used for the study. The rats were purchased from the Department of Biochemistry, Faculty of Natural Sciences, Salem University, Lokoja, Nigeria and housed in the Animal House Facility of the Faculty of Natural Sciences, Ahmadu Bello University, Zaria, Nigeria. They were maintained at room temperature under natural light/dark cycle, and housed in clean cages with saw dust beddings. The rats were allowed access to food and water ad libitum, except when experimental protocol required otherwise.

2.1.2. Drugs and chemicals. Glimepiride (Sonafi Aventis D-65926 Germany), nifedipine (Lek Pharmaceuticals, Slovenia), streptozotocin (Sigma-Aldrich MO.63103 USA), 10% dextrose in water (Juhel Nig Ltd, Enugu), chloroform (Sigma-Aldrich), Polyethylene glycol (PEG) (Sigma-Aldrich) 40% formaldehyde solution (Sigma-Aldrich), ethanol (Sigma-Aldrich).

2.1.3. Instruments and other materials. UV-VIS spectrophotometer (752 China), 92471 Ugo Basile Analgesy Meter, Italy, (AE240 dual range, Metler instrument corporation, USA), glucometer (Accu-chek Active, Roche Diagnostics, Germany), glucose test strips (Accu-chek Active, Roche Diagnostics, Germany), cotton wool, weighing balance dissecting kit, anticoagulant free vacutainers, Cannula, syringes.

2.2. Methods

2.2.1. Induction of diabetes. Diabetes was induced in 12 hours fasted rats by a single intraperitoneal injection of 60 mg/kg streptozotocin, which was reconstituted in a freshly prepared 0.1 M cold citrate buffer, with a pH of 4.5 [16]. After diabetes induction, the rats were fed with 10% dextrose in water for 24 hours to prevent hypoglycaemia that may result from acute massive pancreatic release of insulin [17]. Diabetes was confirmed 7 days after streptozotocin administration, by measuring levels of fasting blood glucose by the means of glucometer and test strips. Rats with fasting blood glucose ≥ 200 mg/dl were thereafter grouped for the study.

2.2.2. Experimental design

40 rats were divided into 5 groups of 8 rats each and were treated orally for 21 continuous days as shown below

   **Group 1:** Non-diabetic control group; treated with PEG and H2O 1 ml/kg

   **Group 2:** Diabetic control group; treated with PEG and H2O 1 ml/kg

   **Group 3:** Diabetic standard group; treated with glimepiride 10 mg/kg at 2000 hrs

   **Group 4:** Diabetic experimental group; treated with glimepiride 10 mg/kg at 2000 hrs + nifedipine 20 mg/kg at 2000 hrs

   **Group 5:** Diabetic experimental group; treated with glimepiride 10 mg/kg at 2000 hrs + nifedipine 20 mg/kg at 0800 hrs

2.2.3. Evaluation of FBG and RBG. Fasting blood glucose was measured following 12 hours of fasting on days 0, 7, 14 and 21 at 0730 hrs using a glucometer. Similarly, random blood glucose was measured periodically within the period of study (on days 0, 6, 10, 13 and 20) at 1300 hrs with a glucometer and test strips.
**Body weight** The rats were weighed on days 0, 7, 14 and 21 with a weighing balance to determine any changes in body weight.

2.2.4. Evaluation of peripheral neuropathy

**Paw-pressure test** Increased sensitivity to pain as a result of probable destruction of nociceptors was determined using the Ugo Basile Analgesy Meter, according to the method of Randell and Selitto \[18\]. The paw pressure test was assessed on days 0, 7, 14 and 21 by applying a force to the rats’ paw, (which was placed on a small plinth under a cone-shaped pusher with a rounded tip, which does not hurt the animal) at a rate of 64 g/s. The nociceptive threshold was defined as the force, in grams, at which a rat struggled to withdraw its hind-paw. The mean nociceptive thresholds at which the rats withdrew their paws were recorded and analyzed. A cut off pressure of 450 g was adapted to avoid mechanical damage to the paw skin.

**Tail immersion test** Thermal hyperalgesia was evaluated on days 0, 7, 14 and 21 in the rats using the hot water tail immersion. The tail of each animal was dipped in a beaker containing hot water maintained at 45.5 ± 0.5°C monitored by a thermometer. The tail flicking responses of rats were observed and the time taken to flick tail was recorded. Three tests separated by at least 10 minutes were performed for each rat, and mean value for each rat was recorded analyzed.

2.2.5. Assessment of retinopathy. Markers for diabetic retinopathy were evaluated as described by \[19\]. At the end of the treatment period (21 days), the animals were euthanized with chloroform and blood samples were collected via the jugular vein. The blood samples collected were centrifuged at 2200 rpm for 10 minutes. The serum obtained was used for the determination of serum magnesium, cholesterol and triglyceride using Randox® diagnostic kits and spectrophotometer.

**Histological study of the retina** The retinal tissues were prepared as described by Arthur and John, \[20\]. Isolated eyes were fixed in 10% formalin until they were processed. The eyes were dehydrated through graded ethanol steps and xylene, and were then embedded in paraffin. Specimens were cut in sections of 6 μm in thickness using a microtome and stained by hematoxyline-eosin (H&E). The stained samples were observed under a microscope and observed for histological changes.

2.2.6. Data analysis. Results are expressed as mean ± standard error of mean (for normally distributed data) and as mean ranks (for data that are not normally distributed). Data obtained were presented as tables and line graphs while histological findings were presented as photomicrographs. One way analysis of variance (ANOVA), followed by Hochberg post hoc test was used in the analysis of single point data that were normally distributed, while Kruskal-Wallis, followed by Dunn-Bonferoni post hoc test was used in analysis of single point data that were not normally distributed. Split plot ANOVA followed by Bonferoni post hoc test was used in the analysis of data collected over time. Results were considered significant at p ≤ 0.05.

3. Results

Effect of 21-day chronomodulated nifedipine administration on glycaemic control in glimepiride-treated hyperglycaemic rats

### 3.1. Glycaemic control

#### 3.1.1. Fasting blood glucose. Treatment with glimepiride alone at 2000 hrs resulted in significant (p < 0.01) reduction in the fasting blood glucose levels on days 7, 14 and 21 when compared with the pre-treatment values. Concurrent administration of glimepiride and nifedipine at 2000 hrs, resulted in an initial delay in the glucose lowering effect of glimepiride, although a significant (p < 0.01) reduction in the fasting blood glucose was observed on days 14 and 21 when compared to the initial levels. In contrast, administration of glimepiride at 2000 hrs along with nifedipine at 0800 hrs resulted in significant (p < 0.01) reduction in fasting blood glucose in a manner similar to that of glimepiride alone at 2000 hrs, except that the reduction in the fasting blood glucose for this group was observed from day 3 as shown in Figure 1.

#### 3.1.2. Random blood glucose. Treatment of diabetic rats with glimepiride alone at 2000 hrs resulted to reduction in the fasting blood glucose for this group was observed from day 3 as shown in Figure 2.

#### 3.1.3. Body weight. Data on the body weight shows significant (p < 0.01) increase in body weight on days 7, 14 and 21 for the non-diabetic control group when compared to the values on day zero. There was no significant change in body weight in the diabetic control group in the successive weeks when compared to initial values, but they were significantly (p < 0.05) lower when compared to the non-diabetic control on days 14 and 21. Treatment with glimepiride alone at 2000
Figure 1: Effect of 21-day chronomodulated nifedipine administration on FBG in glimepiride-treated hyperglycaemic rats. Data are mean ± SEM, b = p ≤ 0.01 compared to non-diabetic control,** = p ≤ 0.01 compared to day zero,## = p ≤ 0.01 compared to diabetic control, split plot ANOVA and Bonferroni post hoc test, n = 5-8, Glim2000hrs+Nife0800hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs; Glim2000hrs+Nife2000hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs.

Figure 2: Effect of 21-day chronomodulated nifedipine administration on RBG in glimepiride-treated hyperglycaemic rats. Data are mean ± SEM, b = p ≤ 0.01 compared to non-diabetic control, *= p ≤ 0.05 compared to day zero, ** = p ≤ 0.01 compared to diabetic control, split plot ANOVA and Bonferroni post hoc test, n = 5-8, Glim2000hrs+Nife0800hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs; Glim2000hrs+Nife2000hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs.

3.2. Effect of 21-day chronomodulated nifedipine administration on peripheral neuropathy in glimepiride-treated hyperglycaemic rats

3.2.1. Paw pressure test. Results from the paw pressure test established the occurrence of peripheral neuropathy at the end of the first week of treatment manifested by significantly (p < 0.05) shorter paw withdrawal latency for all the diabetic groups when compared to the non-diabetic control. Treatment with glimepiride alone at 2000 hrs, and concurrent administration of glimepiride and nifedipine at 2000 hrs produced a significant (p < 0.05) increase in body weight on days 14 and 21 when compared to the initial values. In contrast, concurrent administration of glimepiride and nifedipine at 2000 hrs did not produce any significant difference in body weight when compared to initial values. However, treatment with glimepiride at 2000 hrs and nifedipine at 0800 hrs revealed significant (p < 0.01) increase in body weight on days 7, 14 and 21 when compared to the initial values in a similar manner with the glimepiride alone 8 pm treated group. The result is shown on Table 1.
Table 1: Effect of 21-day chronomodulated nifedipine administration on body weight in glimepiride-treated hyperglycaemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control</td>
<td>118.60 ± 2.11</td>
<td>132.20 ± 3.91*</td>
<td>140.20 ± 4.23**</td>
<td>152.60 ± 5.74**</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>115.20 ± 5.30</td>
<td>123.20 ± 5.30</td>
<td>114.40 ± 4.60a</td>
<td>114.00 ± 4.24a</td>
</tr>
<tr>
<td>Glimepiride 2000 hrs</td>
<td>113.20 ± 3.70</td>
<td>118.80 ± 4.35</td>
<td>126.00 ± 6.32a*</td>
<td>130.20 ± 5.1a*</td>
</tr>
<tr>
<td>Glim2000hrs+Nife2000hrs</td>
<td>124.60 ± 3.55</td>
<td>129.00 ± 3.80</td>
<td>130.80 ± 4.18</td>
<td>132.40 ± 5.71</td>
</tr>
<tr>
<td>Glim2000hrs+Nife0800hrs</td>
<td>120.60 ± 3.66</td>
<td>129.60 ± 5.85*</td>
<td>140.00 ± 4.56a*</td>
<td>129.89 ± 7.95a*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM, * = p ≤ 0.05 compared to day zero, ** = p ≤ 0.01 compared to day zero, a = p ≤ 0.05 compared to diabetic control, split plot ANOVA and Bonferroni post hoc test, n = 5-8, Glim2000hrs+Nife0800hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs; Glim2000hrs+Nife2000hrs = treated with glimepiride at 2000 hrs and nifedipine at 2000 hrs.

2000 hrs did not produce any significant difference in paw withdrawal latency on day 14 and 21 when compared to the initial values. However, treatment with glimepiride at 2000 hrs and nifedipine at 0800 hrs revealed a significant (p < 0.05) and progressive increase in paw withdrawal latency on days 14 and 21 when compared to initial values as shown on Table 2.

3.2.2. Tail immersion test. The tail immersion test results established the occurrence of peripheral neuropathy at the end of the first week of treatment manifested by a significantly (p < 0.05) shorter tail flick latency for all the diabetic groups when compared to the non-diabetic control. Treatment with glimepiride alone at 2000 hrs, and concurrent administration of glimepiride and nifedipine at 2000 hrs did not produce any significant change in tail flick latency in the successive weeks when compared to the initial values. However, treatment with glimepiride at 2000 hrs and nifedipine at 0800 hrs revealed a significant (p < 0.05) and progressive increase in tail flick latency on days 14 and 21 when compared to initial values as shown in Table 3.

3.3. Effect of 21-day chronomodulated nifedipine administration on retinopathy in glimepiride treated hyperglycaemic rats

3.3.1. Serum ocular markers. The serum magnesium levels for the diabetic control group was significantly (p < 0.05) lower than the non diabetic control. Treatment with glimepiride alone at 2000 hrs, and concurrent administration of glimepiride and nifedipine at 2000 hrs did not reveal any significant difference in serum magnesium level when compared to the diabetic control group. However, treatment with glimepiride at 2000 hrs and nifedipine at 0800 hrs revealed significantly (p < 0.05) higher serum magnesium levels when compared to the diabetic control group.

The serum cholesterol and triglyceride levels for the diabetic control groups were significantly (p < 0.05) lower than the non diabetic control. Treatment with glimepiride at 2000 hrs, and concurrent administration of glimepiride and nifedipine at 2000 hrs did not reveal any significant difference in serum cholesterol and triglyceride levels when compared to the diabetic control group. However, treatment with glimepiride at 2000 hrs and nifedipine at 0800 hrs revealed a significantly (p < 0.05) lower serum cholesterol and triglyceride levels when compared to the diabetic control group.

3.3.2. Histology of the retina. Examination of retinal sections of diabetic control rats showed severe distortion of the retinal layers (B). Representative photomicrographs of the retina of rats treated with glimepiride alone at 2000 hrs showed necrosis of the rods and cones and the outer nuclear layer cells (C). Rats treated with concurrent administration of glimepiride and nifedipine at 2000 hrs revealed slight necrosis of the inner and outer layer cells (D) while the representative photomicrograph of the retina of the of rats treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs shows normal histo-architectural retinal layers (E) similar to that of the non-diabetic control group (A).

4. Discussion

A comparison of the glycaemic control data as well as that on the prognosis of peripheral neuropathy and retinopathy obtained from the nifedipine groups treated at 2000 hrs and 0800 hrs, explains how time of drug administration is an important determinant in the outcome of known and potential drug interactions. This is because time of drug administration often affects pharmacokinetics and predicts pharmacodynamic outcomes [4]. The impairment of glycaemic control following concurrent administration of glimepiride and nifedipine at 2000 hrs is a consequence of opposing action of both drugs on a common L-type calcium channels (LTCCs). While glimepiride mediates the closure of K$_{ATP}$ channels [21] through its action on the sulphonylurea receptors to trigger the opening of voltage
dependent LTCCs and insulin release [22], nifedipine binds to LTCCs with high affinity, and selectively blocks them, resulting in inhibition of insulin release [23]. This action affected the glucose lowering property of glimepiride which manifested as a delay in the anti-diabetic effect of glimepiride in this group with consequent worsening of microvascular complications. However, administration of both drugs with at least 12 hrs interval, considering the circadian variation in glucose rhythm [24] and the diurnal variation in the pharmacokinetics of nifedipine, preserved the therapeutic benefit of both drugs. This was evident by the presence of adequate glycaemic control as well as the amelioration of microvascular complications for rats in this group. Studies on nifedipine’s pharmacokinetics in rats showed that the area under the plasma concentration-time curve from zero to six hours [AUC (0-6h)] and the peak plasma concentration [C(max)] after a single oral dose at 0800 hrs was significantly higher with shortest time taken to reach [C (max)] and T (max) in comparison to rats receiving same single dose at 1600 hrs and 0000 hrs [25]. An earlier study also reported that the bioavailability of nifedipine was lost by 40% after evening administration when compared to morning dosing [4]. This is as a result of circadian variation in gastric emptying which is higher during the early morning hours than in the afternoon [27].

Peripheral neuropathy is one of the most common early complications of diabetes mellitus [28] and is characterized by clinical features like allodynia and hyperalgesia due to elevated nociceptive response [29]. In this study, peripheral neuropathy was observed after seven days. This is consistent with previous findings [30] which reported that hyperalgesia due to peripheral neuropathy manifests after seven days in diabetic rats. Data from both the paw pressure and tail immersion tests revealed that nifedipine ameliorates diabetes induced peripheral neuropathy. This is so because reduced nerve blood flow is implicated in peripheral neuropathy. Nifedipine, a vasodilator, acts directly on vascular smooth muscles to bring about increase in blood flow and consequently, prevents nerve conduction deficits in experimental diabetes [12]. It is noteworthy that the amelioration in peripheral neuropathy offered by nifedipine was only significant when nifedipine was administered at 0800 hrs with glimepiride at 2000 hrs. This is due in part to the fact that nifedipine administration was timed to match the rhythm of endogenous vasodilation with approximate highest plasma concentrations of the drug. Endogenous vasodilation peaks during the rest period [31]; suggesting a synergy

Table 2: Effect of 21-day chronomodulated nifedipine administration on mechanical hyperalgesia in glimepiride-treated hyperglycaemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control</td>
<td>130.87 ± 3.52</td>
<td>121.87 ± 7.88</td>
<td>119.87 ± 6.89</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>77.14 ± 2.51</td>
<td></td>
<td>42.71 ± 3.49***</td>
</tr>
<tr>
<td>Glimepiride 2000 hrs</td>
<td>67.57 ± 5.85##</td>
<td>53.42 ± 5.88##</td>
<td>48.42 ± 3.47##</td>
</tr>
<tr>
<td>Glim2000hrs+Nife2000hrs</td>
<td>89.00 ± 5.24##</td>
<td>71.80 ± 8.81##</td>
<td>58.20 ± 4.40##</td>
</tr>
<tr>
<td>Glim2000hrs+Nife0800hrs</td>
<td>65.40 ± 9.96##</td>
<td>82.40 ± 3.68##</td>
<td>98.20 ± 1.22##</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. * = p ≤ 0.05 compared to day seven, ** = p ≤ 0.01 compared to day seven, ## = p ≤ 0.05 compared to non-diabetic control, *** = p ≤ 0.01 compared to diabetic control, split plot ANOVA and Bonferroni post hoc test, n = 5-8,Glim2000hrs+Nife0800hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs; Glim2000hrs+Nife2000hrs = treated with glimepiride at 2000 hrs and nifedipine at 2000 hrs

Table 3: Effect of 21-day chronomodulated nifedipine administration on thermal hyperalgesia in glimepiride-treated hyperglycaemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control</td>
<td>7.89 ± 0.42</td>
<td>7.75 ± 0.52</td>
<td>7.75 ± 0.45</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>5.28 ± 0.71##</td>
<td>3.42 ± 0.36##</td>
<td>1.71 ± 0.35##</td>
</tr>
<tr>
<td>Glimepiride 2000 hrs</td>
<td>3.42 ± 0.31##</td>
<td>3.28 ± 0.35##</td>
<td>2.42 ± 0.29##</td>
</tr>
<tr>
<td>Glim2000hrs+Nife2000hrs</td>
<td>3.80 ± 0.91##</td>
<td>3.40 ± 0.40##</td>
<td>2.40 ± 0.40##</td>
</tr>
<tr>
<td>Glim2000hrs+Nife0800hrs</td>
<td>3.00 ± 0.31##</td>
<td>5.20 ± 0.80##</td>
<td>5.60 ± 0.50##</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. * = p ≤ 0.05 compared to day seven, ** = p ≤ 0.01 compared to day seven, ## = p ≤ 0.01 compared to non-diabetic control, *** = p ≤ 0.05 compared to diabetic control, split plot ANOVA and Bonferroni post hoc test, n = 5-8,Glim2000hrs+Nife0800hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs; Glim2000hrs+Nife2000hrs = treated with glimepiride at 2000 hrs and nifedipine at 2000 hrs
Table 4: Effect of 21-day chronomodulated nifedipine administration serum renal markers in glimepiride-treated hyperglycaemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mg$^{2+}$ (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control</td>
<td>2.38 ± 0.12</td>
<td>65.21 ± 12.55</td>
<td>5.00</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1.08 ± 0.21*</td>
<td>187.97 ± 6.77**</td>
<td>18.20*</td>
</tr>
<tr>
<td>Glimepiride 2000 hrs</td>
<td>1.51 ± 0.13</td>
<td>158.74 ± 8.74*</td>
<td>17.00</td>
</tr>
<tr>
<td>Glime2000hrs+Nife2000hrs</td>
<td>1.65 ± 0.26</td>
<td>145.12 ± 5.86*</td>
<td>18.20</td>
</tr>
<tr>
<td>Glim2000hrs+Nife0800hrs</td>
<td>2.35 ± 0.16*</td>
<td>63.68 ± 5.57##</td>
<td>6.60*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM and mean rank (Triglyceride) * = p ≤ 0.05 compared to non-diabetic control, ** = p ≤ 0.01 compared to non-diabetic control, a = p ≤ 0.05 compared to diabetic control, b = p ≤ 0.01 compared to diabetic control. One-way ANOVA followed by Bonferroni post hoc test for Mg$^{2+}$ and Cholesterol, Kruskal Wallis and Dunn-Bonferroni post hoc test for triglyceride, n = 5-8. Glime2000hrs+Nife0800hrs = treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs; Glim2000hrs+Nife2000hrs = treated with glimepiride at 2000 hrs and nifedipine at 0000 hrs.

Figure 3: A: Photomicrograph of retina section of non-diabetic rat treated with PEG+H$_2$O for 21 days, showing normal histo-architectural retinal layers with rods and cones (RC), outer nuclear layer (ONL), inner nuclear layer (INL), ganglion cell layer (GCL) and optic nerve fibers (ONF) H&E $\times$ 100. B: Photomicrograph of retina section of diabetic rat treated with PEG+H$_2$O 21 days, showing distortion of the retinal layers (RD) H&E $\times$ 100. C: Photomicrograph of retina section of diabetic rat treated with glimepiride at 8 pm for 21 days, showing slight necrosis of the rods and cones (RC) and outer layer cells (ONL) H&E $\times$ 100. D: Photomicrograph of retina section of diabetic rat treated with glimepiride 8 pm and nifedipine 8 pm for 21 days, showing slight necrosis of the inner (INL) and outer layer (ONL) cells H&E $\times$ 100. E: Photomicrograph of retina section of diabetic rat treated with glimepiride 8 pm and nifedipine 8 am for 21 days, showing normal retinal layers with rods and cones (RC), outer nuclear layer (ONL), inner nuclear layer (INL), ganglion cell layer (GCL) and optic nerve fibers (ONF) H&E $\times$ 100.
between endogenous and nifedipine mediated vasodilation. This will ultimately result to enhanced blood supply to peripheral nerves.

Similarly, from the results of serum ocular markers and retina histology, retinopathy appears to have been ameliorated leading to near normal histology and biomarker levels of the group treated with glimepiride at 2000 hrs and nifedipine at 0800 hrs. Here, histological findings showed normal histo-architectural features similar to the non-diabetic control group. Serum magnesium level has been used as a diagnostic marker for retinopathy [31]. The exact mechanism by which magnesium plays a role in diabetic retinopathy is poorly understood, though it has been shown that hypomagnesaemia may contribute to diabetic retinopathy by reducing the rate of inositol transport, abnormal platelet function with subsequent reduced blood and nutrients supply to the retina [31]. One other mechanism involved in the development of retinopathy is occlusion or complete damage of the microvessels supplying the retina [32]. Thus lipid lowering therapies have been shown to be beneficial in diabetic retinopathy [33]. Amelioration of both peripheral neuropathy and retinopathy following a 21 day administration of nifedipine at 0800 hrs alongside glimepiride at 2000 hrs could be as a result of the time of administration of nifedipine. This is because absorption of nifedipine is faster [25], with least first pass effect [4] during the morning time. This suggests that the drug gets to its site of action in high enough concentration to bring about the observed pharmacodynamic outcome.

5. Conclusion

The concurrent administration of glimepiride at 2000 hrs with nifedipine at 0800 hrs improved the prognosis of microvascular complications without affecting glycaemic control. This suggests that the diurnal variation in nifedipine’s pharmacokinetics can be effectively used to advantage, resulting in glimepiride and nifedipine being successfully co-administered without losing the glucose lowering benefits of glimepiride.

Competing Interests

The authors declare no competing interests.

References


