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## Section D: Clinical Pharmacy & Pharmacology



### Sildenafil and Vinpocetine Promote Wound Healing in Diabetic Rats

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#### ABSTRACT

**Objectives:** Many diabetic patients develop foot ulcer that might affect gait, quality of life and may lead to amputation. The present study was designed to investigate the possible effect of phosphodiesterase inhibitors (sildenafil 10 mg/kg/day and vinpocetine 5 mg/kg/day) on healing of experimentally-induced diabetic foot wound in comparison to glimepiride (0.5 mg/Kg/day). **Methods:** All treatments were started on day 15 after streptozotocin (STZ) injection and continued daily for 8 weeks. After 4 weeks of treatment, a wound of fixed size was induced in the dorsal surface of right foot and wound healing was followed throughout the study. Behavior tests (foot print and hot plate test) were performed every 2 weeks. At the end of the study, animals were euthanized, serum insulin, glucose, HbA1C, IL6 and adiponectin were quantified and H& E stained sections (from right foot) were investigated. **Results:** Diabetes delayed wound healing, elevated blood glucose, HbA1c, IL-6, reduced serum insulin and adiponectin. While, treatment with sildenafil, vinpocetine and glimepiride ameliorated most of these effects. **Conclusions:** In conclusion, phosphodiesterase inhibitors as sildenafil, and vinpocetine were effective in ameliorating some of the deleterious effects on diabetes on wound healing indicating that they could be used with other standard therapies for the management of diabetic foot ulcer (DFU).

**Keywords:** Phosphodiesterase inhibitors; Diabetic; Wound; Healing

#### INTRODUCTION

Diabetic vascular complications are a major cause of morbidity and mortality<sup>1</sup>. Many diabetic patients complain from distal, symmetric and sensorimotor neuropathy termed diabetic peripheral neuropathy (DPN)<sup>2</sup> that affects gait and cold / heat sensation<sup>3</sup>.

In diabetes, persistent hyperglycemia is associated with immune dysregulation and prolonged inflammation causing transformation of wounds into non-healing chronic ulcers<sup>4</sup>, which are more common in patients with neuropathic sensory loss<sup>5</sup>.

The beneficial effects of nitric oxide (NO) on wound healing as potentiation of clotting, scavenging of oxidative stress components, improvement of angiogenesis and tissue remodeling have been previously shown<sup>6,7</sup>.

Signaling by nitric oxide (NO) is mediated by cGMP, which is synthesized by guanylyl cyclases and broken down by phosphodiesterases (PDEs)<sup>8</sup>. Therefore, inhibitors of PDEs are associated with increased intracellular cGMP<sup>9</sup> and improved NO signaling.

The present study was designed to investigate the possible effect of two phosphodiesterases inhibitors; sildenafil: PDE-5I<sup>10,11</sup> and vinpocetine: PDE-11<sup>12</sup> on

diabetic foot ulcer induced in rats compared to the standard antidiabetic drug glimepiride.

## MATERIAL AND METHODS

### Animals

Adult male Wistar albino rats (150 – 200 g) were used in this study. Animals were supplied from the national research center (NRC, Dokki, Cairo, Egypt). Experimental design and animal handling procedures were approved by the Ethical Committee for Animal Handling at Zagazig University (ECAHZU) (P2-5-2014).

### Drugs

Streptozotocin (STZ) was purchased from Sigma-Aldrich (Dorset, UK), sildenafil was supplied from Eva pharm (Egypt), vinpocetine was supplied from Amriya pharm (Egypt), and Glimepiride was supplied from MUP (Egypt).

### Induction of diabetes

After 12 hours fasting, rats received a single intraperitoneal injection of STZ (50 mg/kg) dissolved in ice cold distilled water immediately before use<sup>13,14</sup>. Rats were given glucose (10% in drinking water) in the day following STZ injection to avoid acute hypoglycemia. Diabetes was confirmed after 2 weeks and rats with non-fasting blood glucose level between 300 and 400 mg/dl were included in the study.

### Experimental design

On day 15 after induction of diabetes by STZ, diabetic rats were randomly distributed among five experimental groups (n=8): control group; Diabetic, Sildenafil (10 mg/kg/day)<sup>15</sup>, Vinpocetine (5 mg/kg/day)<sup>16</sup> and Glimepiride (0.5 mg/Kg/day)<sup>17</sup>. The required dose of each drug was dissolved in distilled water just before use and treatment continued for 8 weeks by oral gavage.

Animal behavior was tested after 2, 4, 6 and 8 weeks and a wound of fixed size was induced in the dorsal surface of right foot on the 5th week of the study.

### Behavioral tests

#### Hot plate test

Acute thermal pain was modeled by the hot plate which is a method designed to measure thermal analgesia in rodents. The time (in seconds) between the placement of the rats on the plate and the onset of shaking, paw licking and/or jumping off the plate was recorded as the response latency<sup>18</sup>.

#### The footprint test

This test measures latent motor deficits through comparing stride length, gait base width, stride

variability<sup>19</sup>. Briefly, the hind and forepaws of each rat were painted with different colors and the rat was placed at the start of a polyvinyl tunnel (10\*10\*50 cm ending in a dark box) covered with white paper and allowed to pass to the end box (containing food pellets) leaving footprints on the paper. Then the footprints were analyzed to determine the stride length (distance between 2 consecutive prints), gait base of support (distance perpendicular to parallel right and left paw prints), stride variability (difference between shortest and longest stride length) and intrastep distance (distance between alternate right and left paw prints).

### Wound induction

On the day of wound induction (5<sup>th</sup> week), each rat was anesthetized with an intraperitoneal injection of 50 mg/kg thiopental sodium. A rectangular pattern was marked on the dorsal surface of the right foot using a flexible transparent plastic template, and then a layer of skin in full thickness with standard area of 2mm x 5mm was removed and initial wound size was measured on the next day and then measured every 4 days to the end of the study<sup>20</sup>.

### Blood and tissue sampling

Twelve hours after the last injection, body weight was measured and blood was collected from the retro-orbital plexus and centrifuged (3000×g, 4°C, 20 min) to separate serum that was divided into aliquots and stored at -20°C for biochemical analysis. For each animal, the right foot was dissected and fixed in 10% phosphate buffered formalin for histopathological examination.

### Biochemical analysis

Serum insulin level was assayed using ELISA kit (Millipore, Cairo, Egypt)<sup>21</sup>. Blood glucose was determined by glucose meter (Bionime GmbH) using noble metal electrode strips<sup>22</sup>. Glycated hemoglobin was measured using chromatographic spectrophotometric ion exchange BioSystems S.A.® kits (Costa Brava 30, Barcelona, Spain)<sup>23</sup>. Serum IL-6 level was determined using IBL-AMERICA rat IL-6 ELISA Kit<sup>24</sup>. Serum adiponectin level was assayed using CHEMICON® rat adiponectin ELISA kit (Chemicon International, Temecula, CA, USA)<sup>25</sup> according to the manufacturer's protocol.

### Histopathological examination

Hoformalin solution at room temperature. For the histological examinations, paraffin-embedded tissue sections (4 micron) of foot were stained with hematoxylin and eosin (H&E) as previously described<sup>26</sup> and examined under light microscope.

**Table 1. Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on foot print test parameters**

Parameter / group	Time (weeks)			
	2	4	6	8
<b>Gait base (cm)</b>				
Control	3.85±0.20	3.72±0.14	3.33±0.17	3.33±0.11
Diabetic	2.05±0.16*	2.05±0.15*	2.26±0.13*	2.23±0.13*
Sildenafil	3.82±0.18#	3.65±0.16#	3.86±0.11#	3.65±0.14#
Vinpocetine	2.96±0.19#	3.30±0.20#	3.70±0.20#	3.73±0.18#
Glimepiride	3.33±0.27#	3.44±0.25#	3.28±0.27#	3.45±0.26#
<b>Intrastep distance (cm)</b>				
Control	6.74± 0.14	7.00±0.29	7.34±0.23	7.69±0.30
Diabetic	4.11±0.18*	4.38±0.22*	4.58±0.28*	4.91±0.27*
Sildenafil	6.72±0.29#	6.86±0.24#	6.91±0.35#	6.91±0.24#
Vinpocetine	7.28±0.25#	7.04±0.30#	6.61±0.26#	7.14±0.32#
Glimepiride	6.57±0.19#	6.63±0.41#	7.04±0.37#	6.16±0.21#
<b>Left stride length (cm)</b>				
Control	13.72± 0.19	13.66±0.15	13.59±0.18	13.40±0.09
Diabetic	11.79±0.16*	11.92±0.19*	11.87±0.19*	12.03±0.17*
Sildenafil	11.53±0.10	11.59±0.17	11.75±0.17	11.61±0.15
Vinpocetine	11.88±0.16	11.81±0.17	12.01±0.14	12.08±0.14
Glimepiride	11.88±0.22	12.06±0.20	12.26±0.18	12.46±0.19
<b>Right stride length (cm)</b>				
Control	11.90± 0.18	12.08±0.16	12.29±0.19	12.54±0.15
Diabetic	10.80±0.17*	11.03±0.19*	11.44±0.19*	11.69±0.16*
Sildenafil	10.26±0.10	10.81±0.18	11.22±0.23	11.83±0.24
Vinpocetine	11.31±0.08	11.51±0.14	11.73±0.18	11.60±0.19
Glimepiride	10.95±0.19	11.50±0.08	11.92±0.19	12.14±0.18
<b>Left stride variability (cm)</b>				
Control	4.48± 0.10	4.98±0.14	4.72±0.17	4.48±0.10
Diabetic	1.63±0.11*	1.68±0.09*	1.60±0.09*	1.56±0.13*
Sildenafil	4.46±0.22#	4.60±0.12#	4.72±0.11#	5.38±0.06#
Vinpocetine	3.88±0.19#	3.98±0.21#	4.08±0.08#	4.34±0.21#
Glimepiride	2.70±0.12#	2.83±0.22#	3.16±0.16#	2.80±0.17#
<b>Right stride variability (cm)</b>				
Control	4.87± 0.11	4.63±0.24	4.73±0.22	4.70±0.13
Diabetic	1.68±0.20*	1.93±0.21*	1.66±0.16*	1.88±0.15*
Sildenafil	4.40±0.23#	4.26±0.11#	4.58±0.17#	5.00±0.18#
Vinpocetine	3.72±0.17#	3.82±0.19#	4.12±0.18#	4.04±0.22#
Glimepiride	3.76±0.34#	3.75±0.20#	3.60±0.15#	3.81±0.14#

Data are expressed as mean ± S.E.M. n=8. \* significantly different from control, # significantly different from diabetic group at  $p < 0.05$  using two-way ANOVA followed by Bonferroni post hoc test.

## Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5.0<sup>®</sup> software (Graphpad Software, La Jolla, CA; USA). Results were expressed as the mean  $\pm$  standard error of the mean (mean  $\pm$  SEM). One-way analysis of variance (ANOVA) was used for multiple comparison between groups followed by Newman-Keul's post hoc test with level of significance  $p < 0.05$ . Two-way ANOVA followed by Bonferroni post hoc was used to compare statistical difference in behavioural experiments and wound healing.

## RESULTS

### Effect of diabetes and treatment with, sildenafil, vinpocetine and glimepiride on latency time in hot plate test

Diabetes resulted in a significant decrease in hot plate latency time after 2, 4, 6 and 8 weeks of treatment reaching 7.1, 6.1, 6.5, 5.6 vs 46.2, 45, 45.5, 45.4 sec respectively compared to control group. While, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant increase in hot plate latency after 2, 4, 6 and 8 weeks reaching (20, 21, 25, 29), (27.7, 23.6, 24.5, 29.3) (32.8, 31, 30.8, 33.8) vs 7.1, 6.1, 6.5, 5.6 sec respectively compared to diabetic group (**Figure 1**).

### Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on foot print test parameters

**Table 1** shows the changes observed in the foot print test. Diabetes resulted in a significant decrease in gait base after 2, 4, 6 and 8 weeks of treatment reaching 2, 2, 2.2, 2.2 vs 3.8, 3.7, 3.3, 3.3 cm respectively compared to control group. While, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant increase in gait base after 2, 4, 6 and 8 weeks reaching (3.8, 3.6, 3.8, 3.6), (2.9, 3.3, 3.7, 3.7), (3.3, 3.4, 3.2, 3.4) vs. 2, 2.2, 2.2 cm respectively compared to diabetic group.

In addition, diabetes resulted in a significant decrease in intrastep distance after 2, 4, 6 and 8 weeks reaching 4.1, 4.3, 4.5, 4.9 vs 6.7, 7, 7.3, 7.6 cm respectively compared to control group, while, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant increase in intrastep distance after 2, 4, 6 and 8 weeks reaching (6.7, 6.8, 6.9, 6.9), (7.2, 7, 6.6, 7.1), (6.5, 6.6, 7, 6.1) vs. 4.3, 4.5, 4.9 cm respectively compared to diabetic group.

Diabetes also resulted in a significant decrease in left and right leg stride length after 2, 4, 6 and 8 weeks reaching (11.7, 11.9, 11.8, 12) and (10.8, 11, 11.4, 11.6) vs (13.7, 13.6, 13.5, 13.4) and (11.9, 12, 12.2, 12.54) cm respectively compared to control group and this was not

changed following treatment with sildenafil, vinpocetine and glimepiride.

Diabetes resulted in a significant decrease in left stride variability after 2, 4, 6 and 8 weeks reaching 1.6, 1.6, 1.6, 1.5 vs 4.4, 4.9, 4.7, 4.4 cm respectively compared to control group, while, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant increase in left stride variability after 2, 4, 6 and 8 weeks reaching (4.4, 4.6, 4.7, 5.3), (3.8, 3.9, 4, 4.3), (2.7, 2.8, 3.1, 2.8) vs. 1.6, 1.6, 1.5 cm respectively compared to diabetic group.

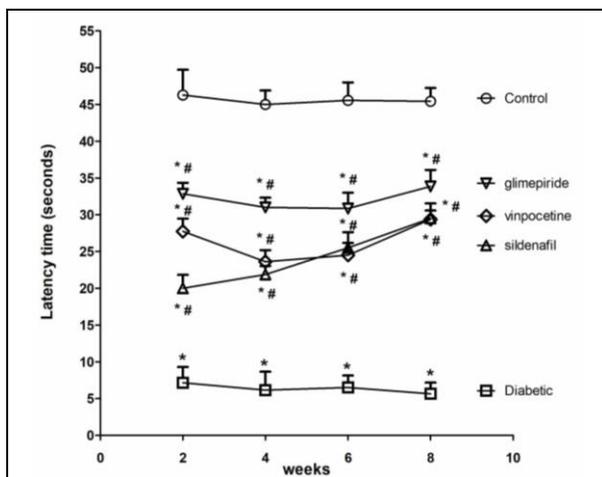
Diabetes resulted in a significant decrease in right stride variability after 2, 4, 6 and 8 weeks reaching 1.6, 1.9, 1.6, 1.8 vs 4.8, 4.6, 4.7, 4.7 cm respectively compared to control group, while, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant increase in right stride variability after 2, 4, 6 and 8 weeks reaching (4.4, 4.2, 4.5, 5), (3.7, 3.8, 4.1, 4), (3.7, 3.7, 3.6, 3.8) vs 1.6, 1.9, 1.6, 1.8 cm respectively compared to diabetic group.

### Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on wound healing

Diabetes resulted in delayed wound healing on days 5, 9, 13, 17, 21, 25, 29 reaching 91.5, 83.2, 68.6, 61.6, 53, 40.8, 38.8 vs 48.2, 36.8, 25.8, 21.5, 14, 11.3, 4.8 mm respectively compared to control group. Improvement in wound healing was observed in diabetic animals treated with sildenafil, vinpocetine and glimepiride on days 5, 9, 13, 17, 21, 25, 29 reaching (64.5, 38.3, 25.3, 17.3, 14.1, 11.2, 6.3), (71.7, 48.5, 29.8, 20.6, 14.8, 10.7, 6.2), (68, 50.2, 29.6, 19, 16.6, 14.1, 9.5) mm vs. 91.5, 83.2, 68.6, 61.6, 53, 40.8, 38.8 mm respectively compared to diabetic group (**Figure 2**).

### Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on tissue histopathology

**Figure 3** shows the effect of diabetes and treatment on tissue histopathology; a normal rat skin formed of normal epidermis and underlying dermis with absence of inflammatory cells and granulation tissue is shown in (**Figure 3a**), while diabetic rat skin after injury showed heavy aggregates of inflammatory cells surrounded by thin-walled vascular spaces (**Figure 3b**). Treatment with sildenafil showed thin dermis and numerous thin-walled vascular spaces in the dermis (**Figure 3c**). Photomicrograph of diabetic rat skin treated with vinpocetine was formed of granulation tissue in the dermis containing numerous thin-walled vascular spaces, red blood cells and surrounded by increased collagen bands (**Figure 3d**), while, treatment with glimepiride resulted in hyperplastic epidermis with mild aggregates of inflammatory cells (**Figure 3e**).

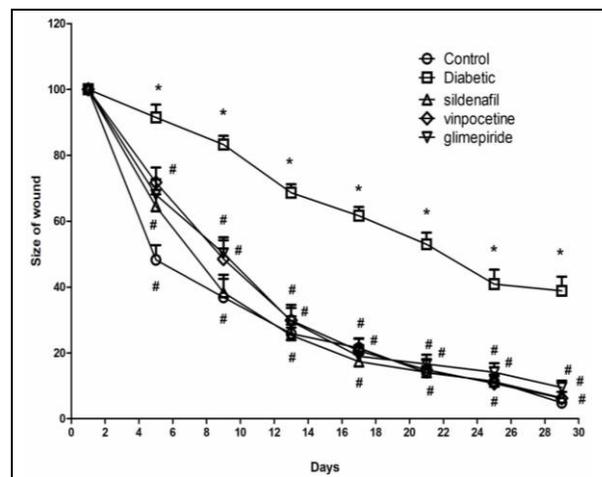


**Figure 1.** Effect of diabetes and treatment with, sildenafil, vinpocetine and glimepiride on latency time in hot plate test. Data are expressed as mean  $\pm$  S.E.M. n=6-8. \* significantly different from control, # significantly different from diabetic group at  $p < 0.05$  using two-way ANOVA followed by Bonferroni post hoc test.

#### Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on body weight, blood glucose, glycated hemoglobin and insulin levels

STZ injection resulted in a significant increase in blood glucose reaching 443.6 vs 103.6 mg.dl<sup>-1</sup> compared to control group, while, administration of sildenafil and glimepiride to diabetic animals resulted in significant decrease in blood glucose reaching 383.4, 116.4 vs 443.6 mg.dl<sup>-1</sup> respectively compared to diabetic group (**Figure 4a**). On the other hand, STZ injection resulted in a significant decrease in insulin level reaching 3.9 vs 16.53  $\mu$ g.l<sup>-1</sup> compared to control group, while, administration of sildenafil and glimepiride to diabetic animals resulted in significant increase in insulin level reaching 6.83, 19.4 vs 3.9  $\mu$ g.l<sup>-1</sup> respectively compared to diabetic group (**Figure. 4b**).

Diabetes also increased HBA1C reaching 13.6 vs 5.4 % compared to control group, while, administration of glimepiride to diabetic animals resulted in significant decrease in HBA1C reaching 7 vs 13.6 % respectively compared to diabetic group (**Figure 4c**). Diabetes resulted in a significant decrease in body weight reaching 110 vs 228 g compared to control group. While, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant increase in body weight reaching 197.2, 159.3, 161.3 vs 110 g respectively compared to diabetic group (**Figure 4d**).



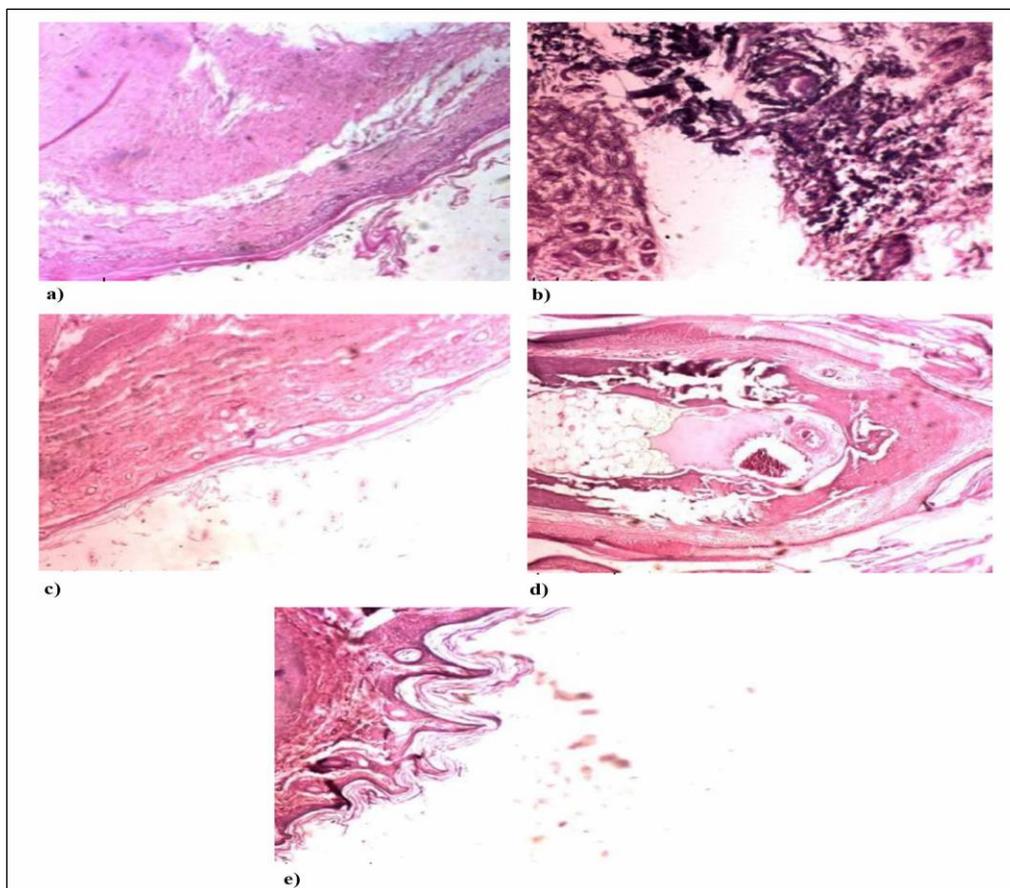
**Figure 2.** Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on wound healing. Data are expressed as mean  $\pm$  S.E.M. n=6-8. \* significantly different from control, # significantly different from diabetic group at  $p < 0.05$  using two-way ANOVA followed by Bonferroni post hoc test.

#### Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on adiponectine and IL-6 level

Diabetes resulted in a significant decrease in serum adiponectin level reaching 3.8 vs 11.5 ng/ml compared to control group, while, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant increase in serum adiponectin level reaching 7.8, 7.1, 9 vs 3.8 ng/ml respectively compared to diabetic group (**Figure 5a**). On the other hand, diabetes resulted in a significant increase in IL-6 level reaching 137.1 vs 24.7  $\mu$ g.l<sup>-1</sup> compared to control group, while, administration of sildenafil, vinpocetine and glimepiride to diabetic animals resulted in significant decrease in IL-6 level reaching 18.4, 43.3, 51.2 vs 137.1  $\mu$ g.l<sup>-1</sup> respectively compared to diabetic group (**Figure 5b**).

#### DISCUSSION

The increased risk of chronic ulcer formation associated with diabetes arises from disruption of wound healing by the pathophysiological abnormalities<sup>27</sup>. Tissue repair and wound healing are complex processes that involve inflammation, epithelialization, angiogenesis, granulation tissue formation, and deposition of interstitial matrix<sup>28</sup>. Diabetes affects the three phases of wound healing; the inflammatory phase through compromising the immune system, the proliferative phase through suppression of angiogenesis and the remodeling phase<sup>29</sup>.



**Figure 3.** Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on tissue histopathology using hematoxylin and eosin staining (H&E): a) control, b) diabetic, c) sildenafil, d) vinpocetine and e) glimepiride.

The current study is the first to investigate the potential protective effect of phosphodiesterase inhibitors on wound healing in diabetic rats.

In the present study, diabetes was induced by a single STZ injection and confirmed by the elevation in glucose level, HBA1c and the reduction in insulin and body weight. Similar effects of STZ were previously shown<sup>30,31</sup>.

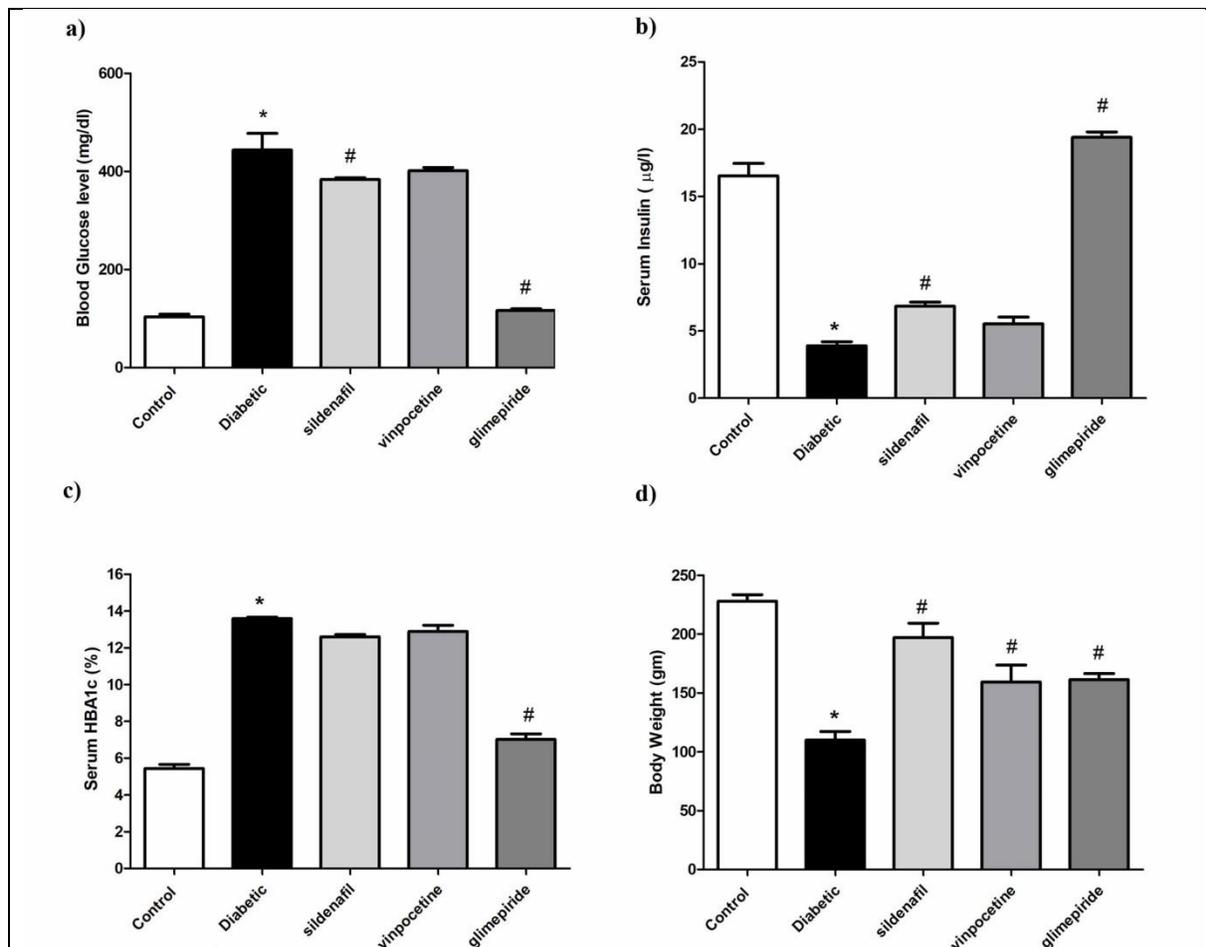
Treatment with the PDE5I sildenafil was associated with improvement in diabetes as evidenced by the significant decrease in glucose level and increased body weight and serum insulin level. Previous studies have demonstrated several beneficial actions of PDE5I in diabetes including prevention of insulin resistance<sup>10</sup>, reduction of HBA1c<sup>32</sup>, modulation of macrovascular, microvascular diabetic complications<sup>33</sup>. While, vinpocetine failed to improve any of the diabetes-related parameters as previously reported<sup>34</sup>. The antidiabetic drug glimepiride normalized blood glucose, HBA1c and serum insulin level as previously described<sup>35,36</sup>.

In the current study, healing of the wound induced in the fifth week was impaired in the diabetic

group, as evidenced by the delay in the healing process and microscopic examination showed decreased epidermal thickness, colour area percentage of collagen and vascular area density compared with the control group.

Previous studies have shown the negative impact of STZ-induced diabetes on healing rate of ulcers in rats<sup>37</sup>. This includes lack of migration of keratinocytes and fibroblasts to diabetic wound associated with reduction of collagen deposition and delayed wound healing<sup>38</sup>. In addition, impaired fibroblast function in diabetic patients was reported<sup>38,39</sup>. In addition, impaired angiogenesis is a clinically significant problem in diabetic patients<sup>40</sup>, and therapies that improve vascularization can improve the outcomes in diabetic wounds and ulcers<sup>41</sup>.

Treatment with sildenafil, vinpocetine and glimepiride accelerated wound healing rate. Light microscopic examination revealed that the newly formed skin was of normal appearance (epidermal thickness, color area percentage of collagen, vascular area density) compared with diabetic group.



**Figure 4: Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on a) blood glucose, b) serum insulin, c) glycated hemoglobin and d) body weight.** Data are expressed as mean  $\pm$  S.E.M. n=6-8. \* significantly different from control, # significantly different from diabetic group at  $p < 0.05$  using One-way analysis of variance (ANOVA) was used for multiple comparison between groups followed by Newman-Keul's post hoc test.

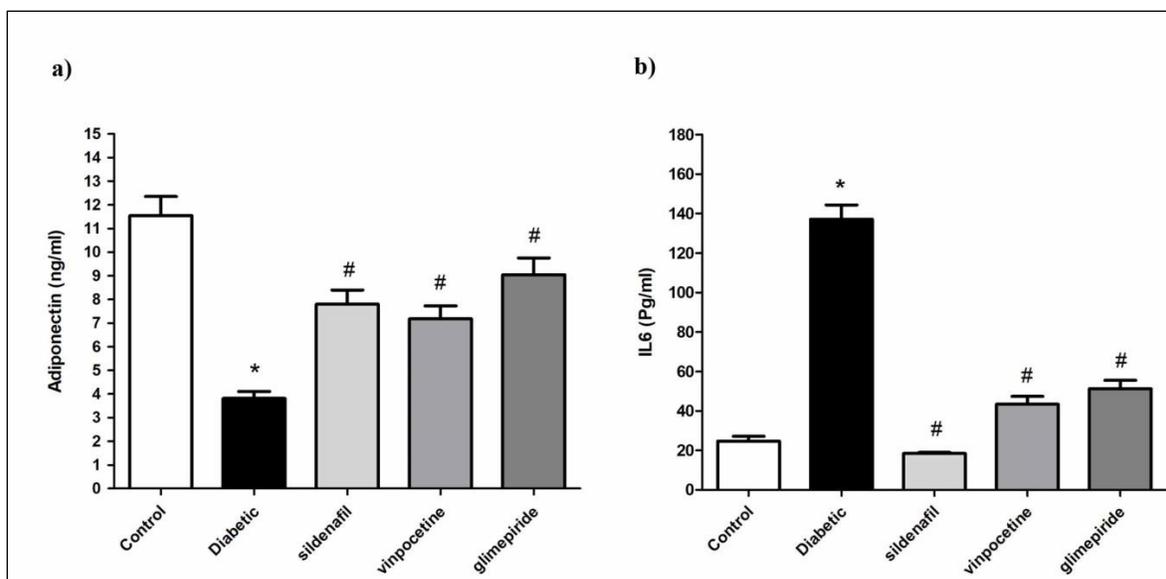
PDE5I caused an improvement of vascular perfusion, tissue blood flow, and vascular density in experimental stroke models<sup>42</sup>. Sildenafil increased regional blood flow and improved motor and sensory conduction velocities in the sciatic nerve and peripheral thermal stimulus sensitivity<sup>43</sup>.

Painful diabetic peripheral neuropathy (PDPN) affects about 25% of type 2 diabetes patients<sup>44</sup>. In addition, STZ-induced diabetes in rats was associated with chemical and thermal hyperalgesia [45]. In the present study, diabetes increased thermal hyperalgesia in the hot plate test as shown previously<sup>46</sup>. While, treatment with sildenafil, vinpocetine and glimepiride increased the latency time in hot plate test indicating a reduction in hyperalgesia induced by diabetes and normal pain perception. Previous studies have shown an anti-nociceptive activity of sildenafil<sup>47, 48</sup>.

Patients with diabetic peripheral neuropathy have slower gait pattern<sup>49</sup>. In the present study, diabetes caused significant change in gait parameters (intrastride distance, stride length, stride variability, gait base). Treatment of rats with sildenafil, vinpocetine and glimepiride caused significant improvement in gait parameters. This is the first study to examine the effect of these drugs on footprint parameters.

Interleukin-6 (IL-6) is a proinflammatory cytokine<sup>50</sup> that was shown to be elevated in patients at higher risk of type 2 diabetes<sup>51</sup>. On the other hand, adiponectin improves insulin sensitivity and exhibits anti-inflammatory and anti-diabetic properties<sup>52</sup>.

In the present study, diabetes increased IL-6 level and lowered adiponectin level. This is in agreement with a previous study that demonstrated higher IL-6 and lower adiponectin plasma levels in diabetic subjects with diabetic foot<sup>53</sup>.



**Figure 5.** Effect of diabetes and treatment with sildenafil, vinpocetine and glimepiride on adiponectin and IL-6 level. Data are expressed as mean  $\pm$  S.E.M. n=6-8. \* significantly different from control, # significantly different from diabetic group at  $p < 0.05$  using One-way analysis of variance (ANOVA) was used for multiple comparison between groups followed by Newman-Keul's post hoc test.

Treatment with sildenafil, vinpocetine and glimepiride increased serum adiponectin and decreased IL-6 level compared to diabetic animals. Previously, sildenafil was reported to exert anti-inflammatory effect on airway inflammation<sup>54</sup>, in diabetes-induced endothelial dysfunction<sup>55</sup> and an *in vitro* cellular model for diabetic neuropathy<sup>56</sup>.

Vinpocetine was shown to exert anti-inflammatory actions against STZ-induced renal damage<sup>57</sup>, cerebral ischemia/reperfusion injury<sup>58</sup> and liver against ischemia-reperfusion injury<sup>59</sup> and similar effects of glimepiride on IL-6 and adiponectin were previously reported<sup>60</sup>.

## CONCLUSION

Our results have shown that the PDE5I sildenafil and the PDE1I vinpocetine were able to ameliorate diabetic foot ulceration induced in rats. These actions were evidenced by suppression of diabetic ulcerative process, improved pain sensation and improvement in wound healing. This is partly attributed to their anti-inflammatory effect and the mild hypoglycemic effect observed with sildenafil. Since the tested drugs are already used clinically, so they could be safely included in diabetic treatment schedule either alone or with current antidiabetic therapies.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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