

## **Partially protective effect of telmisartan on slow healing wounds in diabetic rats: possible role of AGEs-RAGE axis**

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### **ABSTRACT**

Accumulation of advanced glycation endproducts (AGEs) has been found in healthy aging persons and during elevated glucose concentrations. It has been implicated in microvascular and macrovascular damage and delayed wound healing associated with diabetes, presumably through vascular, neurological, or intermediary metabolic modifications. The aim of the present work is to examine the potentially protective effect of telmisartan against diabetes-induced peripheral neuropathy in rats. Diabetic rats (Streptozotocin, STZ 50 mg/kg) were treated with vehicle or telmisartan (5 mg/kg) for 8 weeks. Behavioural tests (footprint and hotplate) were performed every 2 weeks after starting treatment. After 4 weeks of treatment, a wound was induced in the right hindlimb of rats and the wound size was measured every 3 days. At the end of the study, animals were sacrificed, blood, serum and hindlimb tissue were separated and used for further analyses.

STZ treated rats showed a decrease in body weight, insulin, adiponectine level and soluble form of receptor of advanced glycation (sRAGE) expression and an increase in glucose, glycated haemoglobin A1c (HbA1c), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), advanced glycation end products level (AGEs) and receptor of advanced glycation end products (RAGE) expression compared to control group. This was associated with impaired performance in behavior tests and delayed wound healing. Treatment with telmisartan resulted in significant increase in body weight, serum adiponectin, serum sRAGE level and decreased TNF- $\alpha$ , insulin level, serum AGEs and RAGE compared to diabetes. Telmisartan lead to improvement only in gait base and intrastep distance in footprint parameters, while didn't improve hot plate latency or delayed wound healing.

In conclusion, telmisartan may partially improve the deleterious effects of diabetes as peripheral neuropathy through its anti-glycation and anti-inflammatory effects

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**Keywords:** Telmisartan, Diabetes, Neuropathy, advanced glycation end products

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### **INTRODUCTION**

Diabetic peripheral neuropathy (DPN) is the most prevalent complication of diabetes and often manifests as a distal, symmetric, sensorimotor neuropathy. More than half of diabetic patients develop DPN (Harati, 2007). Moreover, 15 - 30% of patients with DPN suffer from painful diabetic neuropathy and the remainder experience a loss of sensation and numbness (Ramos et al., 2007). The clinical symptoms equated with DPN involve poor stride and balance associated with large sensory fibers and abnormal

cold and/or heat sensation associated with small sensory fibers. In addition, hyperalgesia, allodynia, paresthesias, and spontaneous pain is associated with diabetes (Gooch & Podwall, 2004; Edwards et al., 2008). Neuropathic diabetes increases the risk of developing foot ulcers in patients with sensory loss up to seven-fold, compared to non-neuropathic diabetic patients (Wild et al., 2004).

Accumulation of advanced glycation endproduct (AGEs) has been found in healthy aging persons, and this

accumulation is higher during high glucose concentrations and contributes to microvascular and macrovascular complications in diabetes (Luevano-Contreras & Chapman, 2010). AGEs have been implicated in delayed wound healing associated with diabetes, presumably through vascular, neurological, or intermediary metabolic modifications (Peppas et al., 2009). The deleterious effects of AGEs in different tissues are attributed to their chemical, pro-oxidant, and inflammatory actions (Brownlee et al., 1984; Ahmed, 2005). The biological effects of AGEs are exerted by two different mechanisms: the first is receptor-independent through direct damage of protein structures and extracellular matrix metabolism; and the second is through binding to the receptor for advanced glycation end products (RAGE) (Sheetz & King, 2002; Ahmed, 2005). AGE-RAGE interaction activates NAD(P)H oxidase leading to intracellular oxidative stress and activation of NF- $\kappa$ B (nuclear factor kappa beta) (Basta et al., 2005; Lin et al., 2009).

Telmisartan, an angiotensin receptor blocker, is widely used in the treatment of hypertension (Punzi et al., 2013). Telmisartan is the most lipophilic drug of its class and has long half-life after oral administration (approximately 24 hours) (Plosker & White, 2008). In addition, telmisartan was shown to suppress RAGE expression at both mRNA and protein levels in human cultured microvascular endothelial cells (Yamagishi et al., 2008) and in angiotensin-II-exposed endothelial cells (Nakamura et al., 2005). Therefore, the aim of the present work was to examine the potentially protective effect of telmisartan in diabetes-induced peripheral neuropathy through its effect on AGEs-RAGE axis.

## 2. MATERIALS and METHODS

### 2.1. Animals

Adult male albino rats weighing 150–200 g (National research center ,

Cairo, Egypt) were housed in clear polypropylene cages (four rats per cage) and kept on a light–dark cycle of equal duration, under standard environmental conditions. Rats were fed commercially available normal pellet diet and water ad libitum. Experimental design and animal handling procedures were approved by the Ethical Committee for Animal Handling at Zagazig University, (ECAHZU) (P1-5-2014).

### 2.2. Drugs and chemicals

The following drugs and chemicals were used: STZ (Sigma-Aldrich, Dorset, UK), telmisartan (Sigma pharmaceuticals, Egypt), formalin (El-Nasr. Co, Egypt) and dimethyl sulphoxide (DMSO) (Tedia Company Inc., Fairfield, OH).

### 2.3. Study protocol

Animals were randomly divided into 3 experimental groups (8 animals each) as follows: control, diabetic and diabetic treated with telmisartan (5 mg/kg) (Goyal et al., 2011; Zhang et al., 2012). Diabetes was induced by single intraperitoneal (i.p.) +injection of STZ (50 mg/kg) dissolved in ice cold distilled water immediately before use (Reed et al., 1999). The incidence of diabetes was confirmed by measuring blood glucose one week after STZ injection. Animals received tap water and standard diet (20% crude protein, 4% crude fat, 3.5% crude fiber, 6% ash, and 0.5% salt). All drugs were dissolved in dimethyl sulphoxide (DMSO) and treatment started 2 weeks after diabetes induction and continued for 8 weeks. Control and diabetic groups were treated with the vehicle.

### 2.4. Behavioural tests:

The footprint test was used to measure latent motor deficit (Balkaya et al., 2013). Responses of animals in footprint test (stride length, gait base width, stride variability) were measured after 2, 4, 6 and 8 weeks of treatment.

Tunnel of grey Polyvinyl chloride (PVC) with dimensions: 10x10x50cm, ending in a dark box containing food pellets was used. The floor of the tunnel was covered with white paper (2 DIN A4 sheets jointed with tape).

The rat is taken out of the cage and then fixed like for injection. Then paint forepaws and hind paws with a brush with non-toxic finger paint (for children). The mouse gets a little bit stressed by the handling and because of the unknown environment so that it passes the tunnel without exploring it. The mouse is put at the beginning of the tunnel and moves through it into the box, leaving footprints on the paper. Lift the tunnel and take the mouse out for avoiding it turn around and return. Put the mouse back into the cage.

The following parameters can be measured for the resulted footprint paper:

- **Stride length:** which mean the distance between two consecutive left or right paw prints
- **Gait base of support:** mean distance perpendicular to parallel left and right paw prints. This value is determined by measuring the perpendicular distance of a given step to a line connecting its opposite preceding and proceeding steps.
- **Stride variability:** difference between longest and shortest stride length.
- **Intrastep distance:** mean distance between alternate left and right paw prints.

For each step parameter, three values are measured from each run, excluding footprints made at the beginning and end of the run where the animal was initiating and finishing movement, respectively. The mean value of each set of three values is used in subsequent analysis.

The hotplate test was used to measure animal response to thermal stimuli every 2 weeks. The surface of a hot plate (XH-2002 premiere slide warmer; Daigger, Vernon Hills, IL) was heated to  $50 \pm 0.5$

°C. The time (in seconds) between the placement of the rats on the plate and the onset of shaking, paw licking, and jumping off the plate was recorded as the response latency. To avoid tissue damage, 50 seconds was set as the cut-off time after which rats were returned to the cage then retested after 20 minutes (Gardmark *et al.*, 1998).

## 2.5. Wound induction

After 4 weeks of treatment (defined as day 0 of wound), each rat was anesthetized with an intraperitoneal injection of thiopental sodium (50 mg/kg) and a rectangular pattern was marked on the dorsal surface of the right dorsal hindlimb 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 day 1 then every 3 days till the end of the study (Lau *et al.*, 2009).

## 2.6. Biochemical analysis

At the end of the study (12 h after the last injection), 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.

Serum glucose was determined by glucose meter (Bionime GmbH) using noble metal electrode strips (Bimenya *et al.*, 2003). Glycated hemoglobin (HBA1C) was measured using chromatographic spectrophotometric ion exchange BioSystems S.A.® kits (Costa Brava 30, Barcelona, Spain). Serum insulin level was assayed by sandwich ELISA (Millipore, Cairo, Egypt) (Comitti *et al.*, 1987). Serum tumor necrosis factor- $\alpha$ . (TNF- $\alpha$ ) level was determined by ELISA using Quantikine® kit (R&D systems, Cairo, Egypt) (Maskos *et al.*, 1998). Serum adiponectin level was assayed using CHEMICON® rat adiponectin ELISA kit (Chemicon International, Temecula, CA, USA) (Huang *et al.*, 2009).

Serum AGEs level was assayed using MyBioSource MBS700464 rat AGE ELISA kit according to the manufacturer's instructions. Rat serum RAGE was assayed using RayBio® Rat RAGE ELISA kit (RayBiotech, Inc., Norcross, GA, USA) (Nirala *et al.*, 2015). Rat serum soluble receptor of advanced glycation endproduct (sRAGE) was assayed by AVISCERA BIOSCIENCE® ELISA kit (Santa Clara, CA, USA) according to the manufacturer's instructions.

### 2.7. Complete blood count

Complete blood count was determined using automated haematology analyser (Sysmex America Inc., Lincolnshire, IL60069, USA) 3 part differentiation and the following parameters were determined: haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell (RBC), mean corpuscular volume (MCV), haematocrit (HCT), red cell distribution width percentage (RDW%), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), white blood cell (WBC), lymphocyte (LYM), granulocyte (GRAN), minimum inhibitory dilution (MID).

### 2.8. Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5.0® (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 levels of significance;  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ . Two-way ANOVA followed by Bonferroni post hoc was used to compare statistical difference in behavioural experiments and wound healing.

## 3. RESULTS

### 3.1 Effect of diabetes and treatment with telmisartan on body weight, blood glucose, glycated hemoglobin and insulin levels:

Diabetes resulted in a significant decrease in body weight to reach  $111 \pm 5.78$  vs  $203 \pm 4.63$  g compared to control group, while administration of telmisartan to diabetic animals resulted in significant increase in body weight reaching  $154 \pm 4$  vs  $111 \pm 5.78$  g compared to diabetic group (Figure 1(a)).

Diabetes resulted in a significant increase in blood glucose reaching  $404.5 \pm 37.53$  vs  $105.5 \pm 4.92$  mg/dl compared to control group, while administration of telmisartan to diabetic animals didn't result in significant change in blood glucose compared to diabetic group as shown in figure 1(b).

Diabetes resulted in a significant decrease in insulin level reaching  $3.5 \pm 0.25$  vs  $15 \pm 1.03$   $\mu$ g/l compared to control group, while administration of telmisartan to diabetic animals didn't result in significant change in insulin level compared to diabetic group (Figure 1c).

Figure 1(d) showed that diabetes resulted in a significant increase in HbA1C reaching  $13.5 \pm 0.09$  vs  $5.2 \pm 0.24$  % compared to control group, while administration of telmisartan to diabetic animals didn't result in significant change in HbA1C compared to diabetic group

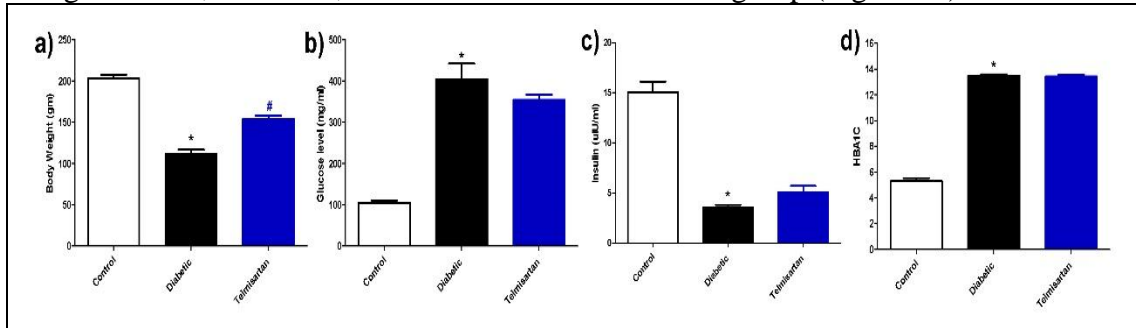
### 3.2 Effect of diabetes and treatment with telmisartan on wound healing:

Diabetes resulted in delayed wound healing on days 5, 9, 13, 17, 21, 25, 29 reaching  $89.7 \pm 2$ ,  $81.8 \pm 1.63$ ,  $76.5 \pm 1.46$ ,  $66.2 \pm 1.22$ ,  $56.7 \pm 1.50$ ,  $40.3 \pm 1.13$  and  $33.5 \pm 0.90$  vs  $50 \pm 1.6$ ,  $33.1 \pm 1.58$ ,  $21.3 \pm 1.05$ ,  $14.8 \pm 0.78$ ,  $12.6 \pm 0.53$ ,  $8.8 \pm 0.47$  and  $4.2 \pm 0.25$  mm<sup>2</sup> compared to control group. For telmisartan; improvement in wound healing was observed only on days 5 and 13 reaching  $83 \pm 1.85$  and  $68.7 \pm 1.22$  vs  $89.7 \pm 2$ ,  $76.5 \pm 1.46$  mm<sup>2</sup> respectively compared to diabetic group (Figure 2 a).

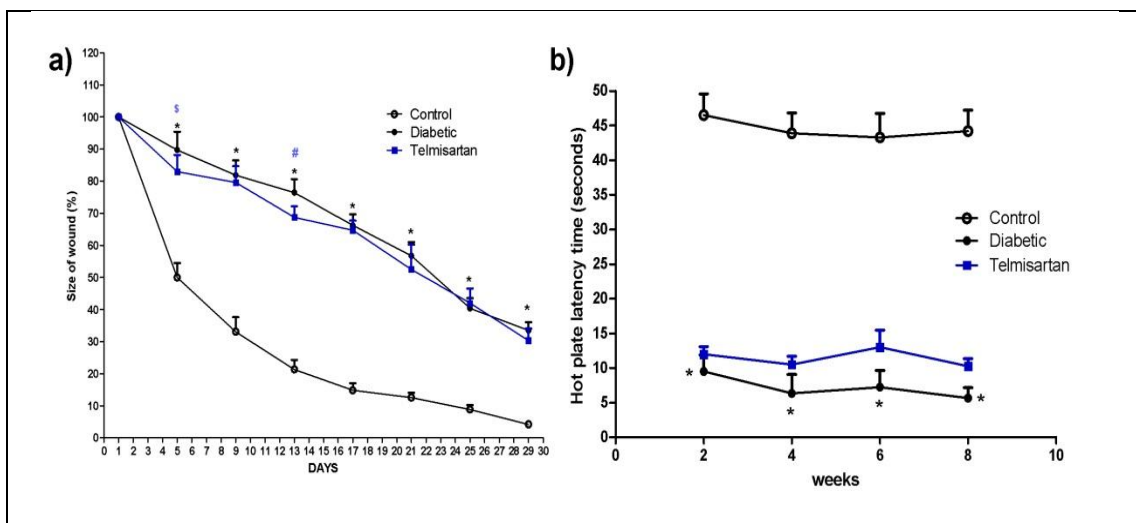
### 3.3 Effect of diabetes and treatment with telmisartan on hotplate test:

Diabetic group showed a significant decrease in hotplate latency time after 2, 4, 6 and 8 weeks of treatment reaching  $9.5 \pm 1.19$ ,  $6.3 \pm 1.11$ ,  $7.2 \pm 0.83$  and

$5.6 \pm 0.61$  sec vs  $46.5 \pm 1.08$ ,  $43.8 \pm 1.04$ ,  $43.2 \pm 1.30$  and  $44.2 \pm 1.35$  sec respectively compared to control group. While telmisartan didn't result in any significant change in hotplate latency compared to diabetic group (Figure 2b).



**Figure 1:** Effect of diabetes induction using STZ (50 mg/kg) and treatment with telmisartan (5 mg/kg) for 8 weeks on: a) body weight, b) blood glucose, c) serum insulin and d) Glycated hemoglobin (HbA1c). Data are presented as mean  $\pm$  SEM (n = 6-8); %, @, \* significantly different from the corresponding control group at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , &, \$, # significantly different from diabetic group at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  using One Way ANOVA and Newman Keuls post hoc test.



**Figure 2:** Effect of diabetes induction using STZ (50 mg/kg) and treatment with telmisartan (5 mg/kg) for 8 weeks on a) wound healing and b) hot plate latency. Data are presented as mean  $\pm$  SEM (n = 6-8); %, @, \* significantly different from the corresponding control group at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , &, \$, # significantly different from diabetic group at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  using Two Way ANOVA and bonferroni post hoc test.

### 3.4 Effect of diabetes and treatment with telmisartan on foot print test:

Diabetes resulted in a significant decrease in gait base after 2, 4, 6 and 8 weeks of treatment reaching  $2.1\pm 0.21$ ,  $2.1\pm 0.15$ ,  $2.5\pm 0.15$  and  $2.6\pm 0.20$  vs  $3.6\pm 0.19$ ,  $3.6\pm 0.23$ ,  $3.6\pm 0.24$  and  $3.9\pm 0.19$  cm respectively compared to control group, while administration of telmisartan to diabetic animals resulted in significant increase in gait base after 2, 4, 6 and 8 weeks reaching  $3.7\pm 0.10$ ,  $3.7\pm 0.20$ ,  $3.6\pm 0.21$  and  $3.5\pm 0.24$  vs.  $2.1\pm 0.21$ ,  $2.1\pm 0.15$ ,  $2.5\pm 0.15$  and  $2.6\pm 0.20$  cm respectively compared to diabetic group (Figure 3 a).

Diabetes resulted in a significant decrease in intrastep distance after 2, 4, 6, 8 weeks of treatment reaching  $4\pm 0.18$ ,  $4.3\pm 0.26$ ,  $4.4\pm 0.30$  and  $4.8\pm 0.31$  vs  $6.7\pm 0.16$ ,  $7\pm 0.34$ ,  $7.2\pm 0.23$  and  $7.3\pm 0.31$  cm respectively compared to control group. While administration of telmisartan to diabetic animals resulted in significant increase in intrastep distance after 2, 4, 6 and 8 weeks compared to diabetic group reaching  $6.8\pm 0.40$ ,  $6.6\pm 0.31$ ,  $6.5\pm 0.37$  and  $6.6\pm 0.55$  vs.  $4\pm 0.18$ ,  $4.3\pm 0.26$ ,  $4.4\pm 0.30$  and  $4.8\pm 0.31$  cm for diabetic group respectively as shown in figure 3 (b).

Induction of diabetes resulted in significant decrease in left stride length after 2, 4, 6 and 8 weeks of treatment reaching  $12.1\pm 0.17$ ,  $12.1\pm 0.20$ ,  $12.1\pm 0.22$  and  $11.8\pm 0.10$  vs  $13.2\pm 0.17$ ,  $13.2\pm 0.14$ ,  $13.4\pm 0.23$  and  $13.4\pm 0.16$  cm respectively compared to control group. While telmisartan failed to change the left stride length compared to diabetic group (Figure 3 c).

Figure 3 (d) showed that diabetes resulted in a significant decrease in right stride length after 2, 4, 6 and 8 weeks of treatment reaching  $10.7\pm 0.23$ ,  $11.1\pm 0.21$ ,  $11.3\pm 0.26$  and  $11.4\pm 0.29$  vs  $11.7\pm 0.17$ ,

$12.2\pm 0.19$ ,  $12.4\pm 0.23$  and  $12.6\pm 0.27$  cm respectively compared to control group. On the other hand, telmisartan has no significant effect on right stride length compared to diabetic group.

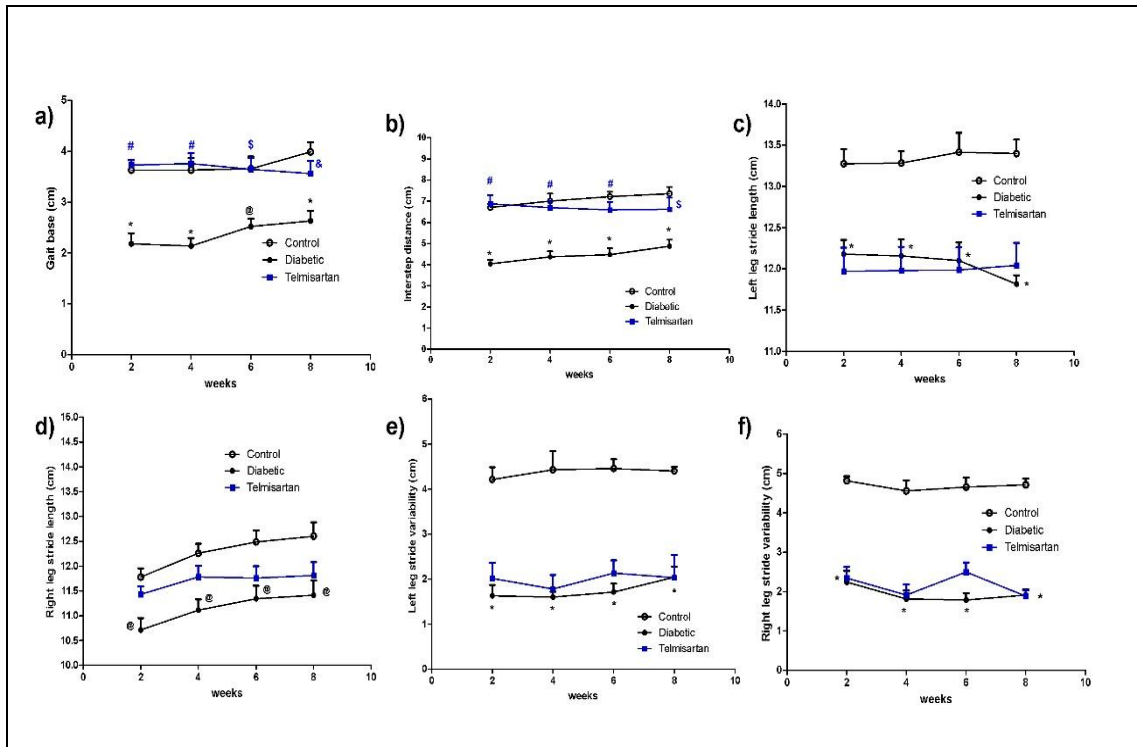
Diabetes resulted in a significant decrease in left stride variability after 2, 4, 6 and 8 weeks of treatment reaching  $1.4\pm 0.13$ ,  $1.6\pm 0.13$ ,  $1.7\pm 0.20$  and  $2.2\pm 0.19$  vs  $4.2\pm 0.27$ ,  $4.4\pm 0.41$ ,  $4.4\pm 0.20$  and  $4.4\pm 0.08$  cm respectively compared to control group, while administration of telmisartan showed no significant change on left stride variability compared to diabetic group as shown in figure 3 (e).

Figure 3 (f) revealed that diabetes resulted in a significant decrease in right stride variability after 2, 4, 6 and 8 weeks of treatment reaching  $2.2\pm 0.28$ ,  $1.8\pm 0.21$ ,  $1.7\pm 0.18$  and  $1.9\pm 0.13$  vs  $4.8\pm 0.11$ ,  $4.5\pm 0.26$ ,  $4.6\pm 0.23$  and  $4.7\pm 0.15$  cm respectively compared to control group, while administration of telmisartan showed no significant change on left stride variability compared to diabetic group.

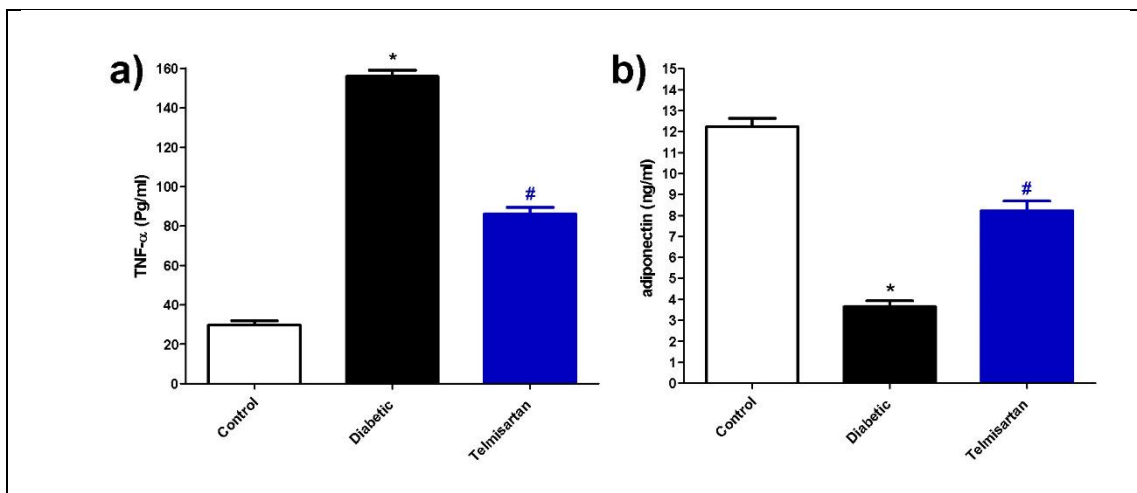
### 3.5 Effect of diabetes and treatment with telmisartan on inflammatory cytokines:

STZ-induced diabetes resulted in significant increase in TNF- $\alpha$  level compared to control group ( $155.8$  vs  $29.7$   $\mu\text{g/l}$ ), while administration of telmisartan to diabetic animals resulted in significant decrease in TNF- $\alpha$  level reaching  $86$  vs  $155.8$   $\mu\text{g/l}$  respectively compared to diabetic group (figure 4(a)).

Diabetes induction resulted in significant decrease in serum adiponectin level reaching  $3.6$  vs  $12.2$  ng/ml compared to control group. Telmisartan resulted in significant increase in serum adiponectin level reaching  $8.2$  vs  $3.6$  ng/ml respectively compared to diabetic group (figure 4 b).



**Figure 3:** Effect of diabetes induction using STZ (50 mg/kg) and treatment with telmisartan (5 mg/kg) for 8 weeks on footprint parameters: a) gait base, b) intrastep distance, c) left leg stride length, d) right leg stride length, e) left leg stride variability and f) right leg stride variability. Data are presented as mean  $\pm$  SEM (n = 6-8); %, @, \* significantly different from the corresponding control group at P<0.05, P<0.01, P<0.001, &, \$, # significantly different from diabetic group at P<0.05, P<0.01, P<0.001 using Two Way ANOVA and bonferroni post hoc test.



**Figure 4:** Effect of diabetes induction using STZ (50 mg/kg) and treatment with telmisartan (5 mg/kg) for 8 weeks on: a) serum TNF- $\alpha$  and b) serum adiponectin levels. TNF- $\alpha$ , tumor necrosis factor- $\alpha$ . Data are presented as mean  $\pm$  SEM (n = 6-8); %, @, \* significantly different from the corresponding control group at P<0.05, P<0.01, P<0.001, &, \$, # significantly different from diabetic group at P<0.05, P<0.01, P<0.001 using One Way ANOVA and Newman Keuls post hoc test.

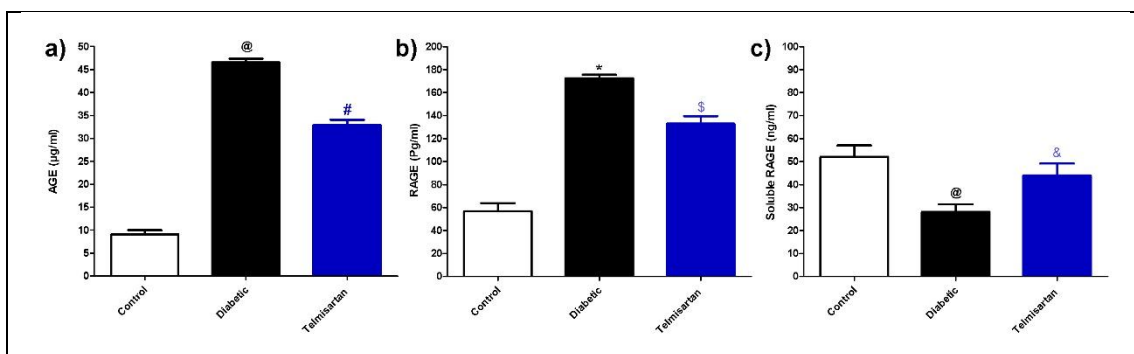
### 3.6 Effect of diabetes and treatment with telmisartan on serum AGEs, RAGE and sRAGE:

In figure 5 (a), diabetes resulted in a significant increase in AGEs level reaching  $46.5 \pm 0.80$  vs  $9.1 \pm 0.87$   $\mu\text{g/ml}$  compared to control group, while administration of telmisartan to diabetic animals resulted in significant decrease in AGEs level reaching  $32.8 \pm 1.18$  vs  $46.5 \pm 0.80$   $\mu\text{g/ml}$  respectively compared to diabetic group. Diabetes induction resulted in a significant increase in RAGE level compared to control group  $172.6 \pm 2.99$  vs  $56.92 \pm 6.95$  Pg/ml. On the other hand, administration of telmisartan to diabetic animals resulted in significant decrease in RAGE level reaching  $132.9 \pm 6.59$  vs  $172.6 \pm 2.99$  Pg/ml respectively compared to diabetic group as shown in figure 5 (b).

Diabetes resulted in a significant decrease in sRAGE level reaching  $28.1 \pm 3.26$  vs  $52 \pm 4.96$   $\mu\text{g/l}$  compared to control group. Treatment with telmisartan resulted in significant increase in sRAGE level reaching  $43.9 \pm 5.22$  vs  $28.1 \pm 3.26$   $\mu\text{g/l}$  respectively compared to diabetic group as shown in figure 5 (c).

### 3.6 Effect of diabetes and treatment with telmisartan on complete blood count:

Table (1) shows that diabetes resulted in a significant decrease in HGB level reaching 10 vs 13.2 g/dl compared to control group, while administration of telmisartan to diabetic animals resulted in significant increase in HGB level reaching 13.2 vs 10 g/dl respectively compared to diabetic group.



**Figure 5:** Effect of diabetes induction using STZ (50 mg/kg) and treatment with telmisartan (5 mg/kg) for 8 weeks on: a) AGEs, b) sRAGE and c) RAGE. AGE, advanced glycation endproduct; sRAGE, soluble receptor of advanced glycation endproduct; RAGE, receptor of advanced glycation endproduct. Data are presented as mean  $\pm$  SEM (n = 6-8); %, @, \* significantly different from the corresponding control group at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , &, \$, # significantly different from diabetic group at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  using One Way ANOVA and Newman Keuls post hoc test.

Diabetes resulted in a significant decrease in MCH level reaching 24.7 vs 40.1 pg compared to control group, while administration of telmisartan to diabetic animals resulted in significant increase in MCH level reaching 38.2 vs 24.7 pg respectively compared to diabetic group as shown in table 1.

Diabetes resulted in a significant decrease in MCHC level reaching 16 vs 18.7 g/dl compared to control group. Treatment with

telmisartan lead to significant increase in MCHC level reaching 18.4 vs 16 g/dl respectively compared to diabetic group (Table 1).

Table 1 shows that diabetes resulted in a significant increase in RBCs level compared to control group reaching  $8.9$  vs  $6.7 \times 10^{12}/\text{L}$ . Telmisartan administration to diabetic animals resulted in significant decrease in RBC level  $7.2$  vs



8.9 x 10<sup>12</sup>/L respectively compared to diabetic group.

Diabetes resulted in a significant increase in MCV level reaching 54.4 vs 46.7 fl compared to control group. While, administration of telmisartan to diabetic animals resulted in significant decrease in MCV level reaching 48.3 vs 54.4 fl respectively compared to diabetic group (table 1).

Table 1 shows that STZ-induced diabetes resulted in a significant increase in HCT level reaching 42.6 vs 33 %

**Table 1:** Effect of telmisartan (5mg.kg<sup>-1</sup>) on HGB, MCH, MCHC, RBC, MCV, HCT and RDW% in diabetes.

Treatment	HGB g/dl	MCH pg	MCHC g/dl	RBC 10 <sup>12</sup> /L	MCV fl	HCT %	RDW% %
<b>Control</b>	13.22 ± 0.79	40.10 ± 0.32	18.74 ± 0.18	6.73 ± 0.28	46.74 ± 0.61	33.04 ± 2.137	12.56 ± 0.13
<b>Diabetic</b>	10.02 ± 0.98 <sup>%</sup>	24.72 ± 1.93 <sup>*</sup>	16.06 ± 0.83 <sup>%</sup>	8.98 ± 0.21 <sup>%</sup>	54.4 ± 2.65 <sup>%</sup>	42.64 ± 1.32 <sup>%</sup>	14.12 ± 0.3 <sup>%</sup>
<b>Telmisartan</b>	13.26 ± 1.022 <sup>&amp;</sup>	38.2 ± 0.58 <sup>#</sup>	18.44 ± 0.44 <sup>&amp;</sup>	7.23 ± 0.64 <sup>&amp;</sup>	48.34 ± 0.92 <sup>&amp;</sup>	34.92 ± 3.08 <sup>&amp;</sup>	12.40 ± 0.25 <sup>&amp;</sup>

<sup>%</sup>P<0.05, <sup>@</sup>P<0.01, <sup>\*</sup>P<0.001, compared with the corresponding control group values;

<sup>&</sup>P<0.05, <sup>\$</sup>P<0.01, <sup>#</sup>P<0.001 compared with the corresponding diabetes group values; by One Way ANOVA and Newman Keuls *post hoc* test

Table 2 shows that diabetes resulted in a significant decrease in PLT number reaching 115.2 vs 296 x 10<sup>9</sup>/L compared to control group. On the other hand, administration of telmisartan to diabetic animals resulted in significant increase in PLT number reaching 452.6 vs 115.2 x 10<sup>9</sup>/L respectively compared to diabetic group.

Induction of diabetes resulted in a significant increase in MPV level reaching 6.9 vs 5.7 fl compared to control group, while administration of telmisartan to diabetic animals resulted in significant decrease in MPV level reaching 5.9 vs 6.9 fl respectively compared to diabetic group as shown in table 2.

compared to control group, while administration of telmisartan to diabetic animals resulted in significant decrease in HCT level reaching 34.9 vs 42.6 % respectively compared to diabetic group.

Diabetes resulted in a significant increase in RDW% reaching 14.1 vs 12.5 % compared to control group. Administration of telmisartan to diabetic animals resulted in significant decrease in RDW% reaching 12.4 vs 14.1 % compared to diabetic group as shown in table 1.

Diabetes induction resulted in a significant increase in PDW level reaching 9.9 vs 8.5 fl compared to control group, while administration of telmisartan to diabetic animals resulted in significant decrease in PDW level reaching 8.2 vs 9.9 fl respectively compared to diabetic group as shown table 2.

Diabetes resulted in a significant increase in PCT level reaching 0.5 vs 0.3 % compared to control group, while administration of telmisartan to diabetic animals resulted in significant decrease in PCT level reaching 0.2 vs 0.5 % respectively compared to diabetic group (Table 2).

**Table 2:** Effect of telmisartan (5mg.kg<sup>-1</sup>) on PLT, MPV, PDW and PCT in diabetes.

Treatment	PLT 10 <sup>9</sup> /L	MPV fl	PDW fl	PCT %
Control	296.0 ± 28.33	5.78 ± 0.1	8.58 ± 0.1	0.36 ± 0.02
Diabetic	115.2 ± 7.41*	6.94 ± 0.24%	9.94 ± 0.25 <sup>@</sup>	0.54 ± 0.01 <sup>%</sup>
Telmisartan	452.6 ± 27.85 <sup>#</sup>	5.98 ± 0.22 <sup>&amp;</sup>	8.22 ± 0.32 <sup>#</sup>	0.26 ± 0.02 <sup>#</sup>

%P<0.05, <sup>@</sup>P<0.01, \*P<0.001, compared with the corresponding control group values;  
&P<0.05, <sup>\$</sup>P<0.01, <sup>#</sup>P<0.001 compared with the corresponding diabetes group values; by One Way ANOVA and Newman Keuls *post hoc* test

Diabetes resulted in a significant increase in WBC level reaching 12.2 vs 10.2 x 10<sup>9</sup>/L compared to control group, while administration of telmisartan to diabetic animals resulted in significant decrease in WBC level reaching 8.4 vs 12.2 x 10<sup>9</sup>/L respectively compared to diabetic group (Table 3).

Diabetes resulted in a significant increase in GRAN level reaching 3.1 vs 1.5 x 10<sup>9</sup>/L compared to control group, while administration of telmisartan to diabetic

animals resulted in significant decrease in GRAN level reaching 1.1 vs 3.1 x 10<sup>9</sup>/L respectively compared to diabetic group (Table 3).

Diabetes induction resulted in a significant increase in MID level reaching 2.1 vs 1.7 x 10<sup>9</sup>/L compared to control group, while administration of telmisartan to diabetic animals resulted in significant decrease in MID level reaching 1.3 vs 2.1 x 10<sup>9</sup>/L respectively compared to diabetic group (Table 3).

**Table 3:** Effect of telmisartan (5mg.kg<sup>-1</sup>) on WBC, LYM, GRAN and MID in diabetes.

Treatment	WBC 10 <sup>9</sup> /L	LYM 10 <sup>9</sup> /L	GRAN 10 <sup>9</sup> /L	MID 10 <sup>9</sup> /L
Control	10.25 ± 0.59	7.38 ± 0.28	1.5 ± 0.14	1.76±0.09
Diabetic	12.22 ± 0.3 <sup>@</sup>	3.92 ± 0.24*	3.12 ± 0.27*	2.16±0.11 <sup>%</sup>
Telmisartan	8.4 ± 0.65 <sup>#</sup>	5.1 ± 0.38	1.12 ± 0.12 <sup>#</sup>	1.30±0.12 <sup>#</sup>

%P<0.05, <sup>@</sup>P<0.01, \*P<0.001, compared with the corresponding control group values;  
&P<0.05, <sup>\$</sup>P<0.01, <sup>#</sup>P<0.001 compared with the corresponding diabetes group values; by One Way ANOVA and Newman Keuls *post hoc* test.

#### 4. DISCUSSION

The aim of the present work was to investigate the potentially protective effect of telmisartan, an angiotensin receptor blocker, on diabetes-induced peripheral neuropathy in rats.

Our data conclusively showed that telmisartan partially improved diabetic neuropathy in rats as evidenced by improving some foot print parameters but lacked beneficial effects on delayed wound healing. The effect of telmisartan might be partly attributed to its anti-inflammatory effects.

Streptozotocin, STZ, is well known to cause damage of pancreatic β-cells

(Szkudelski, 2001; Lenzen, 2008). STZ-diabetic rat model reliably produces many of the signs and symptoms of human diabetes, in particular diastolic cardiac dysfunction, cataracts and neuropathy (Wei *et al.*, 2003). In rats, it has been reported that a dose ranging from 25 to 100 mg/kg STZ was successful in inducing a dose dependent hyperglycemia (Hayashi *et al.*, 2006). In the present study, intraperitoneal injection of single dose of STZ (50 mg/kg) developed significant increase in blood glucose level and significant decrease in serum insulin level as previously described by Hassan *et al.*, (2014).

Measurement of HBA1C in patients with diabetes is accepted as a standard for assessment of glycemic control and is a critical element in clinical practice (Lester, 1989). The three parameters, glucose, HBA1C and insulin play fundamental roles in the pathogenesis of diabetes depending on their levels in circulation (George *et al.*, 2013). In this work, diabetes was associated with elevation of HBA1C. This is consistent with previous work where diabetes induction with STZ for 8 weeks raised HBA1C level (Huang *et al.*, 2003). Telmisartan didn't affect either blood glucose or insulin or HBA1C levels.

Altered glucose homeostasis and persistent hyperglycemia in diabetes lead to AGEs accumulation which are primarily responsible for the damage of cells that have a slow turn over (like neuronal tissue) (Mishra *et al.*, 2008). In the present study, diabetes was associated with elevated serum levels of AGEs, RAGE and a reduction in sRAGE level. Diabetes was previously shown to be associated with higher levels of AGEs (Tanaka *et al.*, 1999) and RAGE (Kislinger *et al.*, 2001) and reduced sRAGE levels (Norata *et al.*, 2009; Devangelio *et al.*, 2007). Telmisartan administration in the present study resulted in reduction of serum levels of AGEs and RAGE and increased sRAGE compared to diabetes. Previously, telmisartan suppressed RAGE expression at both mRNA and protein levels in human cultured microvascular endothelial cells (Yamagishi *et al.*, 2008).

Previous studies have clearly shown that failure of foot ulcer healing can eventually lead to amputation, therefore, diagnosis and treatment of ulcers should be both timely and effective (McLennan *et al.*, 2006). In the present study, diabetes was associated with delayed wound healing which is in consistence with the study carried out by Tong *et al.*, 2012 in which diabetes induction by STZ in rats showed slow rate of healing of the ulcer made on the dorsal skin. While, treatment

with telmisartan caused initial improvement in wound healing after 5 days of wound induction but this improvement started to disappear starting from the 9<sup>th</sup> day post-injury.

Peripheral neuropathy may be asymptomatic, or symptomatic, they may be negative or positive. Negative symptoms include loss of sensation and loss of strength, while positive symptoms include pricking or pain (Davies *et al.*, 2006). Chronic painful diabetic peripheral neuropathy can cause symptoms that last for years and severely impair quality of life (Benbow *et al.*, 1998). Diabetic animals in this study showed elevated thermal hyperalgesia in hotplate test and this is in agreement with previous study of Sharma *et al.*, 2006. In this study, telmisartan didn't prevent thermal hyperalgesia in diabetic animals which is in accordance with the result of Al-Rejaie *et al.* (2015) in which telmisartan failed to inhibit thermal hyperalgesia in a diabetic neuropathic pain model in wistar rats but improved mechanical hyperalgesia using Randall-Selitto test.

Walking capacity and performance decrease with progression of foot complications (Kanade *et al.*, 2006). The footprint test is designed to measure latent motor deficit. It has already been used for detection of ataxia in Huntington disease (Balkaya *et al.*, 2013). In the current study, diabetic rats revealed significant change in foot print parameters. People with diabetic peripheral neuropathy are known to present with a slower gait pattern (Menz *et al.*, 2004). Telmisartan administration improved gait base and intrastep distance only. This is the first study to examine the effect of telmisartan on footprint.

The initiation of the pain process during diabetic neuropathy is mediated through proinflammatory cytokines, such as TNF- $\alpha$  that is released from activated microglia (Zychowska *et al.*, 2013), while adiponectin secreted by adipose tissue possess insulin-sensitizing, anti-inflammatory and antioxidant properties

(Balsan *et al.*, 2015). Low plasma adiponectin levels, might contribute to diabetic and pre-diabetic peripheral and central neuropathy, respectively (Anderson *et al.*, 2014). In the present work, diabetes was associated with raised level of TNF- $\alpha$  and low level of adiponectin is in consistence with the previous study of Tsunekawa *et al.*, 2003. Telmisartan administration reduced the level of TNF- $\alpha$  and raised the level of adiponectin but still significantly different from control group. The present results are also in agreement with the findings reported by Guo *et al.*, 2016 in which the expression of adiponectin and its receptors were upregulated in the testis of STZ-induced diabetic rats after telmisartan treatment.

Diabetic animals in the present study showed an increased HCT which is in consistence with previous study of Tulloch-Reid *et al.*, 2004 in which the level of HCT was associated with higher risk of developing type 2 diabetes. Increasing HCT value is considered to be potentially pathological as shown by the study of Danesh *et al.* (2000), who found that high hematocrit increases cardiovascular risk. Abnormal increase in the size of red blood cells as indicated by increased MCV accompanied with decrease in both MCH and MCHC and increase in RDW% were observed in the present study. Similar results were previously reported (Morse *et al.*, 1981; Hardikar *et al.*, 2012).

Platelets secrete components of the blood coagulation pathway and growth factors necessary for wound healing (Vinik *et al.*, 2001). In the present study, PLT count decreased while, MPV, PDW and PCT levels were significantly increased which is in harmony with previous studies (Demirtas *et al.*, 2015; Yeom *et al.*, 2016). Shortened platelet survival in the diabetic rat is caused initially by a platelet defect (Winocour *et al.*, 1984). The increased platelet activity may play a role in the development of vascular complications of

this metabolic disorder. MPV is an indicator of the average size and activity of platelets. Larger platelets are younger and exhibit more activity (Kodiatte *et al.*, 2012) because of elevated prothrombic contents, such as thromboxane A2, thromboxane B2, platelet factor 4, serotonin, and platelet-derived growth factor (Gasparyan *et al.*, 2011). As with MPV, increased PDW and PCT are also reported to be associated with diabetes and vascular complications (Davi & Patrono, 2007; Demirtas *et al.*, 2015).

Elevated WBC count, an indicator of chronic inflammation, is associated with both macro- and microvascular complications in type 2 diabetes (Tong *et al.*, 2004). In the present study, overall WBC count was elevated in diabetic group. GRAN and MID count was elevated but LYM count was decreased significantly compared to control group. The results of the current study are consistent with previous studies (Turner *et al.*, 1994; Woo *et al.*, 2011; Mahmoud, 2013). Reduced number of blood-circulating lymphocytes was previously found in diabetic patients which is attributed to high occurrence of apoptosis in lymphocytes (Otton *et al.*, 2004).

Telmisartan restored normal level of HGB, MCH, MCHC, RBC, MCV, HCT, RDW%, MPV, PDW, PCT, GRAN and MID but significantly increased the level of PLT and decreased WBC compared to diabetic group but still significantly different from control group. On the other hand, telmisartan didn't affect the level of LYM. Telmisartan was previously shown to lower the level of WBCs in animal model of airways inflammation (Hussain *et al.*, 2014).

## Conclusion

Our results have revealed that targeting AGEs by telmisartan which act by reducing level of AGEs and its receptor RAGE and increasing sRAGE level was partially effective against diabetic neuropathy but ineffective against improving delayed wound healing in

diabetes in the tested dose, however further research is needed to test dose dependency or the effect of longer treatment period on wound healing.

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## التأثير الوقائي الجزئي للتلميسارتان في الاعتلال العصبي المحيطي الناتج عن مرض السكري من خلال تأثيره على محور اج وراج.

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<sup>١</sup>قسم الصيدلة والسموم، كلية الصيدلة، جامعة الزقازيق، ٤٤٥١٩ الزقازيق، مصر  
<sup>٢</sup>قسم الصيدلة، كلية الصيدلة، جامعة تبوك، المملكة العربية السعودية.

تم العثور على تراكم لمنتجات الغليكاين المتقدمة (اج) في الأشخاص الأصحاء المسنين وأثناء تركيزات الجلوكوز المرتفعة. وقد تورط في الأوعية الدموية الدقيقة والأوعية الدموية الكبيرة وتأخر التئام الجروح المرتبطة بالسكري، ويفترض من خلال التعديلات الأيضية الأوعية الدموية، العصبية، أو الوسيط. الهدف من هذا العمل هو فحص التأثير الوقائي المحتمل لـ للتلميسارتان ضد الاعتلال العصبي المحيطي الناتج عن مرض السكري في الجرذان. تم علاج الجرذان المصابة بالسكري بالستريبيوزوتوسين (٥٠ مجم / كجم) بواسطة التلميسارتان (٥٠ مجم / كجم) لمدة ٨ أسابيع. أجريت الاختبارات السلوكية (البصمة وطبق التسخين) كل أسبوعين بعد بدء العلاج. بعد ٤ أسابيع من العلاج، تم إحداث جرح في القدم الخلفي للفئران وتم قياس حجم الجرح كل ٣ أيام. في نهاية الدراسة، تم التضحية بالحيوانات، وتم فصل الدم، مصل الدم والأنسجة القدم الخلفي واستخدامها لمزيد من التحليلات. أدى مرض السكري إلى انخفاض في وزن الجسم والأنسولين ومستوى الاديونيكيتين و صيغة مستقبلة لمستقبل الغليسيشن المتقدم وزيادة في نسبة الجلوكوز و الهيموغلوبين و عامل نخر الورم والمستوى النهائي للمنتجات غلكايشن و مستقبلات منتجات نهاية غلكايشن المتقدمة تعبير مقارنة بمجموعة التحكم. وارتبط هذا مع ضعف الأداء في اختبارات السلوك وتأخر التئام

الجروح. أدى العلاج باستخدام التلميسارتان إلى زيادة معنوية في وزن الجسم ، و اديبونيكيتين المصل ، وانخفاض عامل نخر الورم ، مصل والمستوى النهائي للمنتجات غلكايشن و مستقبلات منتجات نهاية غلكايشن المتقدمة في المصل مقارنة مع مرض السكري. يؤدي للتلميسارتان إلى التحسن فقط في قاعدة المشية ومسافة في بارامترات البصمة ، في حين أنه لم يحسن من تأخر الصفيحة الساخنة أو تأخر التئام الجروح .في الختام ، قد يحسن للتلميسارتان جزئيا من الآثار الضارة لمرض السكري والاعتلال العصبي المحيطي من خلال آثاره المضادة للجليكوزين والمضادة للالتهابات