

Vascular protective actions of the angiotensin receptor blockers valsartan and losartan in STZ-induced diabetic rats

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ABSTRACT

Augmentation of angiotensin II (Ang II) signaling with the subsequent overproduction of the reactive oxygen species (ROS) has been documented to be involved in the development of diabetic cardiovascular complications. This study aimed to assess the potential impact of angiotensin II type 1 (AT1) receptors blockage, with valsartan or losartan, on the progression of the cardiovascular complications associated with diabetes in comparison with the classical antidiabetic drug, metformin. Animals were randomly assigned to one normal group, one group of untreated streptozotocin (STZ) (45 mg/kg)-induced type I diabetic rats, and five groups of STZ diabetic rats that received different daily oral treatments over a 6-week period as follows: metformin (100 mg/kg), valsartan (7 and 14 mg/kg), and losartan (5 and 10 mg/kg). Systolic blood pressure and heart rate were measured weekly. At the end of the study, blood samples were withdrawn for the estimation of the oxidative stress biomarkers, and histopathological assessments of aorta were conducted as well. The STZ-induced diabetic rats exhibited blood pressure elevation, heart rate reductions, disturbances in the oxidative stress biomarkers, as well as aortic histopathological aberration compared with normal control group. Metformin administration resulted in a non-significant tendency to ameliorate the elevated blood pressure with no effect on the heart rate and partially reversed the disrupted oxidative stress biomarkers. However, treatment with valsartan or losartan abolished the development of hypertension in diabetic rats with no effect on the associated bradycardia and successfully attenuated the generation of ROS as compared to the diabetic rats. Treatment with metformin, valsartan, and losartan showed ameliorative effects on the aortic histopathological aberrations observed in diabetic rats. In STZ diabetic rats, our data suggest that the beneficial effects afforded by blockage of the AT1 receptor with valsartan or losartan treatments could be in part mediated through a mechanism that may involve inhibition of ROS production.

INTRODUCTION

Diabetes is a public health problem in both developing and developed countries. Globally, based on the seventh edition of the International Diabetes Federation (IDF) atlas, there were more than 415 million adults diagnosed with diabetes in 2015. This number is projected to increase to 642 million people worldwide by 2040, with type 2 diabetes mellitus (T2DM) accounting for

about 90% of diabetic patients (IDF, 2015; Singh *et al.*, 2011; Xu *et al.*, 2016).

Diabetes mellitus (DM) is a chronic endocrine and metabolic disorder that results from the impairment of the pancreatic insulin secretion due to the destruction of the pancreatic β cells (type 1, insulin-dependent or juvenile onset diabetes) or diminished insulin sensitivity at the tissue level (type II, insulin-independent or maturity onset diabetes) (Mihailovic *et al.*, 2015; Thompson and Godin, 1995). The common outcome of both type I and type II diabetes is the characteristic hyperglycemia associated with its negative consequences on carbohydrate, fat, and protein metabolism (Krejpcio *et al.*, 2011; Yang, 2011).

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Diabetes is known to markedly raise morbidity and mortality rates due to the progressive development of cardiovascular complications. It is associated with a greater incidence of hypertension and atherosclerosis which are considered major risk factors in the development of diabetic cardiomyopathy and stroke (Fiorino *et al.*, 2012; Sasaki *et al.*, 2013).

A strong crosstalk between oxidative stress and hyperglycemia has been documented; oxidative stress is a major contributor in pathogenesis and progression of diabetes and its related cardiovascular complications. On the other hand, hyperglycemia has been proposed to play a pivotal role in inducing the generation of the reactive oxygen species (ROS) in both types of diabetes (Mihailovic *et al.*, 2015).

Hyperglycemia has been shown to increase the incidence of hypertension in humans and in animal models of diabetes. This increase in blood pressure has been attributed to the over activation of the renin-angiotensin system (RAS) with a subsequent production of the vasoconstrictor angiotensin II (Ang II) (Fang *et al.*, 2004; Malfitano *et al.*, 2012). There is a growing body of evidence that the dysregulation of the RAS is a strong promoter for the formation of ROS (Senapaty *et al.*, 2014). In addition, several studies have postulated that Ang II is closely involved in the progression of arterial stiffness, enhancing vascular smooth muscle cell proliferation, and the formation of atherosclerotic plaques independent of its actions on blood pressure (Tikellis *et al.*, 2013; Uehara and Takeda, 2008).

The objective of the present study is to investigate the potential antioxidant and vascular protective actions of long-term treatment with angiotensin receptor blockers (ARBs), namely, losartan and valsartan in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Valsartan and losartan were obtained as gifts from Novartis Pharma and Hikma Pharmaceuticals Co, Cairo, Egypt, respectively. Metformin was obtained as a gift from Amoun Pharmaceuticals Co., Cairo, Egypt and STZ was purchased from Sigma-Aldrich (MO, USA). The chemicals and reagents used in the present study were of analytical grade.

Animals

Male albino Wistar rats (240–270 g) obtained from the animal house, National Research Centre (Cairo, Egypt), were used. The animals were housed in plastic cages covered with wood shavings flooring and they were kept for at least 1 week in the laboratory room prior to testing under standard housing conditions (room temperature 25°C with alternating 12 hours light and dark cycles) and were allowed free access to water and food *ad libitum*, unless otherwise specified. All animals' procedures were performed in accordance to the Institutional Ethics Committee of Faculty of Pharmacy, Cairo University and of the National Research Centre, Egypt and in accordance with the recommendations for the proper care and use of laboratory animals.

Induction of diabetes

Overnight fasting animals were rendered diabetic by a single intraperitoneal (i.p.) injection of 45 mg/kg STZ freshly dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5)

(Lo Giudice *et al.*, 2002). Age-matched control rats were injected with an equal volume of citrate buffer only and served as normal control. For the next 24 hours after STZ injection, the STZ treated animals were supplemented with 5% glucose solution in the drinking bottle *ad libitum* to counteract the initial hypoglycemia and promote survival by avoiding death due to hypoglycemic shock (Chahlia, 2009). Confirmations of the animals' diabetic state were assessed by blood glucose concentrations analysis 72 hours after STZ injection. Animals with a blood glucose level of 250 mg/dl \approx 14 mmol/l or higher (Hansen *et al.*, 2007), as well as with the symptoms of polyuria, polydipsia, and polyphagia were considered diabetic, while animals not meeting these criteria were excluded from this study.

Experimental design

The selected diabetic rats were randomly assigned to six groups in addition to a seventh group (normal control). Each group consisted of 8–10 rats that received oral daily treatments for 6 weeks as follows: Group (i) received only the vehicle i.p. and served as normal rats. Group (ii) received single STZ (45 mg/kg) i.p. and served as a +ve control diabetic rats. Group (iii) diabetic rats received metformin (100 mg/kg/day) orally and served as reference standard (El-Batran *et al.*, 2006). Group (iv) diabetic rats received valsartan (7 mg/kg/day) orally (El-Shenawy *et al.*, 2004). Group (v) diabetic rats received valsartan (14 mg/kg/day) orally (Zhang *et al.*, 2008). Group (vi) diabetic rats received losartan (5 mg/kg/day) orally (Mori *et al.*, 2002). Group (vii) diabetic rats received losartan (10 mg/kg/day) orally (Yavuz *et al.*, 2003).

To avoid the incidence of the lethal ketoacidosis, all diabetic rats received 2 I.U. insulin (Insulatard, Novo Nordisk) daily to maintain a blood glucose level at 300–350 mg/dl (Hansen *et al.*, 2007; Riser *et al.*, 2003).

Systolic blood pressure and heart rate measurements

Systolic blood pressure (SBP) and heart rate of all animals were indirectly measured weekly from the tail of pre-trained conscious rats by the tail cuff technique as described earlier by Fregly, (1961) and El-Mosallamy *et al.*, (2012). This was performed using the non-invasive blood pressure monitor (model ML 125 NIBP, ADInstruments Pty. Ltd., Sydney, Australia). Training was conducted by the method described by Irvine *et al.* (1997). SBP (cuff deflation pressure) was defined as the point at which the cuff pressure corresponds to the restoration of the first caudal artery pulse. The average of at least three measurements was taken at each occasion. Heart rate was recorded automatically by a counter triggered by the pulse wave.

Sample preparation

Twenty-four hours after the last treatment, blood samples were withdrawn from the overnight fasted rats via the retro-orbital vein of each animal under light ether anesthesia (Cocchetto and Bjornsson, 1983) and collected in heparinized tubes. An aliquot of heparinized blood was used for estimating its reduced glutathione (GSH) level. The other aliquot was centrifuged to separate plasma, which was then used for estimation of lipid peroxides level measured as malondialdehyde (MDA).

The animals were then euthanized by decapitation. The aorta was dissected, carefully cleaned of adhering tissues, washed

in saline, and then immediately immersed in 10% neutral buffered formalin for histopathological examination.

Determination of plasma lipid peroxides

Lipid peroxides were estimated in plasma as thiobarbituric acid reactive substances measured as MDA following the method of Mihara and Uchiyama (1978). The concentration of MDA in plasma was expressed as nanomoles per milliliter.

Determination of blood reduced glutathione

Blood GSH was determined according to the method described by Beutler *et al.* (1963). Briefly, this was conducted by spectrophotometric estimation of the yellow color of 5-thio-2-nitrobenzoic acid at 412 nm. This color is produced from the reaction between GSH with 5, 5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB). The results of blood GSH were expressed as milligrams per deciliter.

Histopathological examination

For histopathological investigation, aortic specimens from all animals were dissected immediately after decapitation, washed thoroughly with formal saline, and then fixed in 10% neutral-buffered formal saline for at least 72 hours. All the specimens were washed in tap water for half an hour, dehydrated in ascending grades of alcohol (70%–80%–90% and finally in absolute alcohol), cleared in xylene, impregnated in soft paraffin wax at 55°C, and then embedded in hard paraffin. Serial sections, each 6 µm thick, were cut and stained with hematoxylin and eosin (Drury and Wallington, 1980). The sections were scanned and analyzed by an expert pathologist. Images were captured and processed using Adobe Photoshop version 8.0.

Statistical analysis

In the present study, all results were expressed as mean ± standard error of the mean. All data were subjected to statistical analysis using Statgraphics Centurion XVI version 16.1.11, Stat point, Inc.

Data of SBP and heart rate were analyzed using repeated measures two-way analysis of variance (ANOVA) to test for interaction between time and grouping factors followed by Duncan's multiple range test. Results of blood GSH and lipid peroxidation were statistically analyzed using one-way ANOVA followed by Duncan's multiple range test. A probability of less than 0.05 was used as criterion for statistical significance.

RESULTS

Effects of valsartan and losartan on systolic blood pressure

The mean SBP was 107.4 ± 3.5 mmHg in the normal group, which did not change throughout the experimental period. Diabetes was induced with a single i.p. injection of STZ (45 mg/kg), and the diabetic untreated rats started to show a significant elevation in the SBP by 9.6% during the second week. Starting from the fourth week, the STZ diabetic untreated rats showed a significant sustained increase in the SBP until the end of the experiment by 7.34%, 8.77%, and 15.55%, respectively, as compared to that of the zero time. Additionally, administration of metformin (100 mg/kg/day) showed a milder significant increase

in SBP at the end of experiment by 6.89% compared to their zero time readings.

On the other hand, the daily administration of valsartan (7 and 14 mg/kg) and losartan (5 and 10 mg/kg) significantly protected the STZ-induced diabetic rats from the expected elevation in the SBP (Fig. 1).

Effects of valsartan and losartan on heart rate

The mean heart rate was 308.53 ± 11.57 BPM in the normal group which did not change throughout the experimental period. The heart rate was significantly decreased in the diabetic untreated rats starting from the first week by 10.48%, 13.09%, 13.68%, 5.27%, 1.74%, and 5.14%, respectively, to the end of the experimental period, as compared to their zero time values.

The daily administration of metformin (100 mg/kg), valsartan (7 and 14 mg/kg), and losartan (5 and 10 mg/kg) failed to ameliorate the decrease in heart rate observed in the STZ diabetic rats. Additionally, the heart rate lowering effect of valsartan (7 mg/kg) and losartan (10 mg/kg) was found to be significant when compared with the metformin treated group (Fig. 2).

Effect of valsartan and losartan on plasma MDA and blood GSH levels

Induction of diabetes in rats by the single intraperitoneal injection of STZ significantly increased the level of plasma MDA by 146.11% and significantly decreased the blood GSH level by 21.89% when compared with the normal rats.

The daily administration of metformin (100 mg/kg), valsartan (7 and 14 mg/kg), and losartan (5 and 10 mg/kg) significantly decreased the plasma MDA level by 47.38%, 33.53%, 33.27%, 37.19%, and 29.62%, respectively, as compared with the diabetic group.

Moreover, the daily administration of metformin (100 mg/kg), valsartan (7 mg/kg), and losartan (5 mg/kg) for 6 weeks resulted in numerical non-significant increase in blood GSH level by 13.57%, 11.99%, and 16.63%, respectively, in comparison with the diabetic rats. These results were also non-significant from the normal control rats reaching 88.71%, 87.48%, and 91.10% of the normal control rats' value.

However, administration of valsartan (14 mg/kg) and losartan (10 mg/kg) resulted in a significant increase in the blood GSH level by 32.44% and 27.5%, respectively, as compared to the STZ diabetic rats (Figs. 3 and 4).

Effects of valsartan and losartan in comparison with metformin on rat aortic pathological changes

Light microscopic examination of aortic sections obtained from normal rats showed normal appearance of the aortic structure, with intact tunica intima composed of one layer of intact endothelium, thick tunica media occupying most of the thickness of the vessel wall and appeared full of wavy elastic fibers embedded in it, and thin outer tunica adventitia made from loose connective tissue (Fig. 5).

The aortic sections of control diabetic rats showed abnormal tunica media/adventitia ratio because of thinning of the tunica media with stretching of the elastic fibers in it with noticeable increase in thickness of adventitia (Fig. 6a).

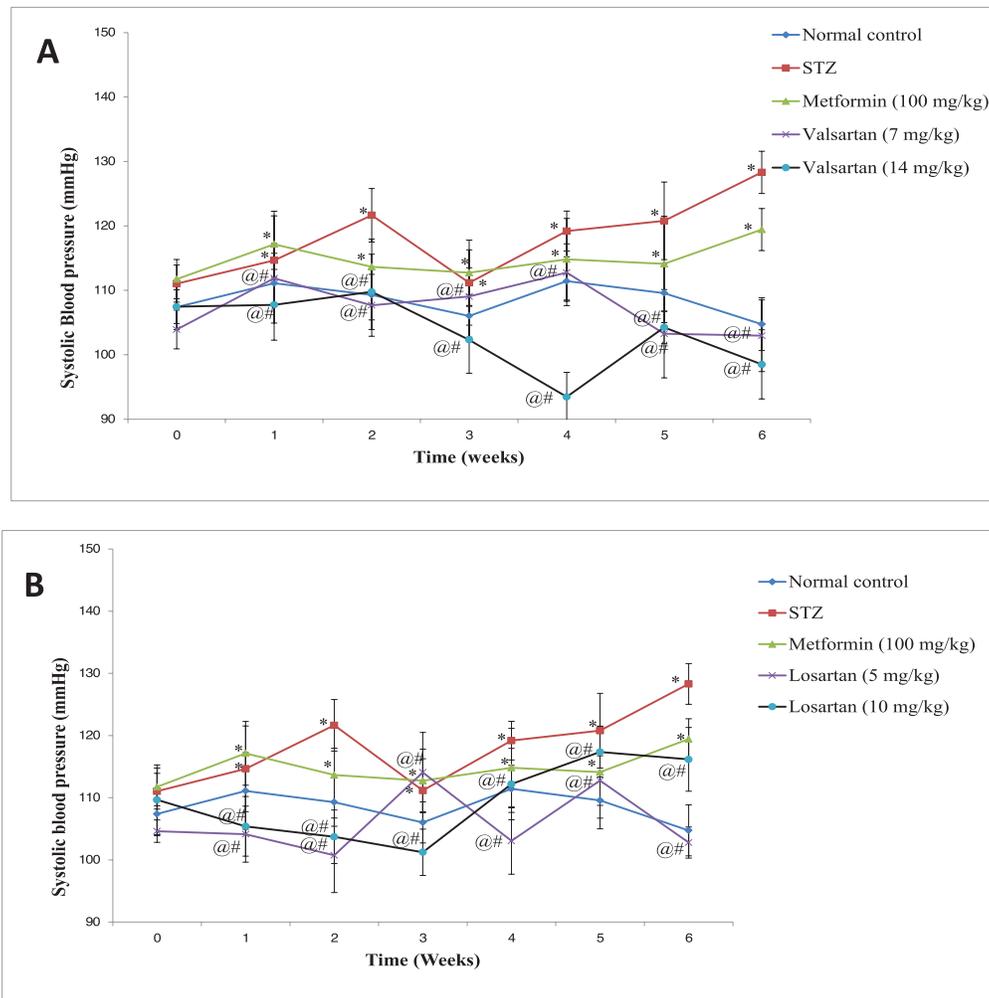


Fig. 1. Effects of (A) Valsartan (7 and 14 mg/kg, P.O.) and (B) Losartan (5 and 10 mg/kg, P.O.) in comparison with metformin (100 mg/kg, P.O.) on SBP in STZ-induced diabetes in rats. Diabetes was induced in rats by single intraperitoneal injection of STZ. Valsartan, losartan, and metformin were orally administered daily for 6 weeks. SBP (mmHg) of animals was indirectly measured each week by the tail-cuff technique. Data are expressed as mean \pm SEM ($n = 6-10$). The statistical comparison of difference between groups was carried out using two-way ANOVA, followed by Duncan's multiple range test. *Statistically significant from normal control group at the corresponding time at $p < 0.05$. @Statistically significant from STZ control group at the corresponding time at $p < 0.05$. #Statistically significant from metformin-treated group at the corresponding time at $p < 0.05$.

Administration of metformin (100 mg/kg) daily for 6 weeks slightly modulated the STZ-induced aortic changes by the regaining of the wavy elastic fibers in tunica media. However, the adventitia is still thicker than normal (Fig. 6b).

The aortic sections of the diabetic rats that received valsartan (7 mg/kg) daily for 6 weeks showed thick adventitia and abnormal-shaped muscle fibers of tunica media with swollen disoriented nuclei, and the elastic fibers regained their wavy appearance (Fig. 6c).

The aortic sections of the diabetic rats that received valsartan (14 mg/kg) daily for 6 weeks showed normalization of the general architecture of the aortic wall although some nuclei of smooth muscle fibers of tunica media were still swollen with abnormal appearance and orientation (Fig. 6d).

Administration of losartan (5 mg/kg) daily for 6 weeks to the diabetic rats failed to ameliorate the hyperglycemic effects

of STZ. The tunica media appeared thinner than normal with interlocking elastic fibers, and the elastic fibers lost their wavy appearance. In addition, the nuclei of smooth muscle fibers appeared swollen and disoriented (Fig. 6e).

On the other hand, aortic tissues of diabetic rats that received losartan (10 mg/kg) daily showed regaining of the normal architecture of the aorta (Fig. 6f).

DISCUSSION

In the present experiments, the single i.p. injection of rats with 45 mg/kg STZ elicited a time-dependent significant elevation of SBP that was associated with a significant decrease in heart rate when compared with the normal rats during the 6 weeks after the induction of diabetes.

These results were in agreement with the studies of Haidara *et al.* (2009) and Hussien (2014). They demonstrated

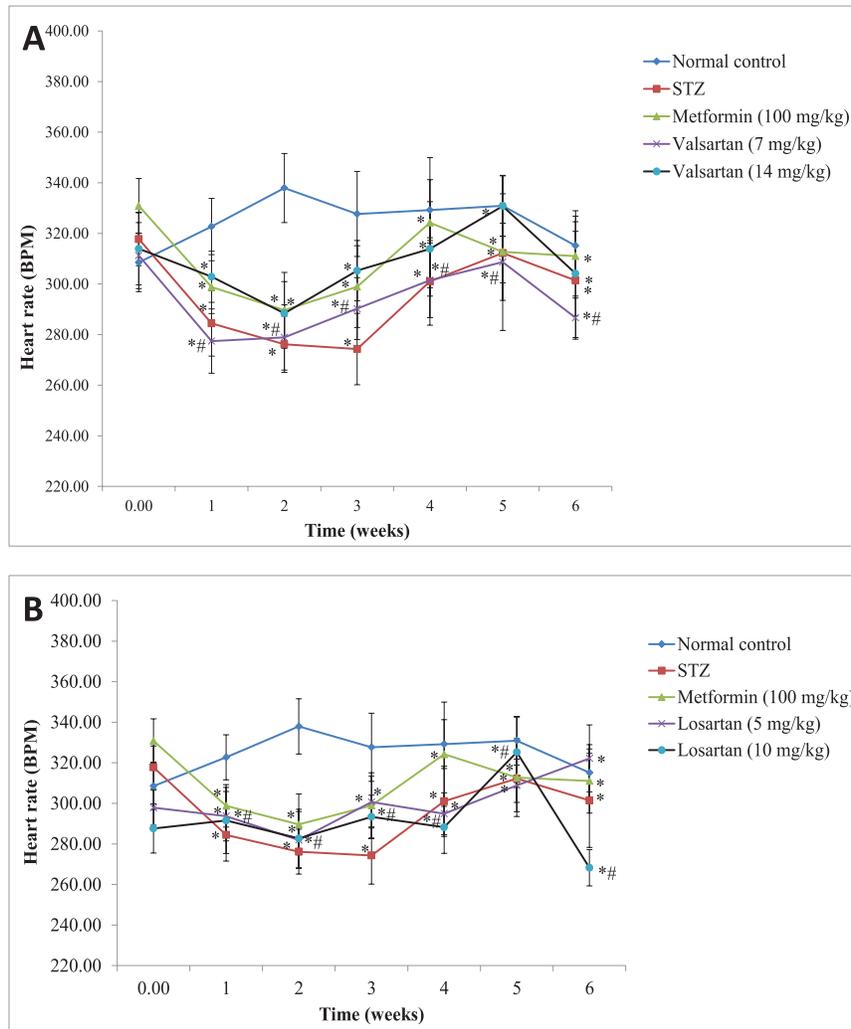


Fig. 2. Effects of (A) Valsartan (7 and 14 mg/kg, P.O.) and (B) Losartan (5 and 10 mg/kg, P.O.) in comparison with metformin (100 mg/kg, P.O.) on heart rate in STZ-induced diabetes in rats. Diabetes was induced in rats by single intraperitoneal injection of STZ. Valsartan, losartan, and metformin were orally administered daily for 6 weeks. Heart rate (BPM) of animals was indirectly measured each week by the tail-cuff technique. Data are expressed as mean \pm SEM ($n = 6-10$). The statistical comparison of difference between groups was carried out using two-way ANOVA, followed by Duncan's multiple range test. *Statistically significant from normal control group at the corresponding time at $p < 0.05$. @Statistically significant from STZ control group at the corresponding time at $p < 0.05$. #Statistically significant from metformin treated group at the corresponding time at $p < 0.05$.

that induction of hyperglycemia by STZ produced marked and sustained hypertension and decreases in heart rate of the experimental rats.

Several mechanisms have been postulated to illustrate the major causative factors involved in the development of hypertension in response to hyperglycemia, including generation of ROS, inflammation, activation of sympathetic nervous system, up-regulation of rennin angiotensin aldosterone system (RAAS), accumulation of advanced glycation end-products (AGEs), as well as impaired arterial vascular integrity with subsequent atherosclerosis, arterial stiffening, and stenosis (Hussien, 2014).

The upregulation of the RAAS with the subsequent increase in the formation of Ang II is one of the major contributing mechanisms involved in the pathogenesis of hypertension under diabetic conditions. Musial *et al.* (2013) reported an increase in

the plasma angiotensin-converting enzyme (ACE) activity in diabetic rats, suggesting augmentation of Ang II synthesis.

Angiotensin II, a potent vasoconstrictor, has emerged as a major mediator of several pathophysiological mechanisms in a number of clinical disorders such as essential hypertension, atherosclerosis, as well as in diabetic cardiovascular damage (Nasser *et al.*, 2014). It is a well-known mitogen involved in vascular smooth muscle cell proliferation, a major causative factor for the accumulation of AGEs (Kurtz and Pravenec, 2008; Wang *et al.*, 2013), and it has been identified as a stimulator for the progression of arterial stiffness (Uehara and Takeda, 2008). The vast majority of the cardiovascular activities of Ang II were attributed to its binding with AT1 receptors, which are widely expressed in the vasculature, heart, kidney, adrenal gland, brain, and liver (Prabhakar, 2013).

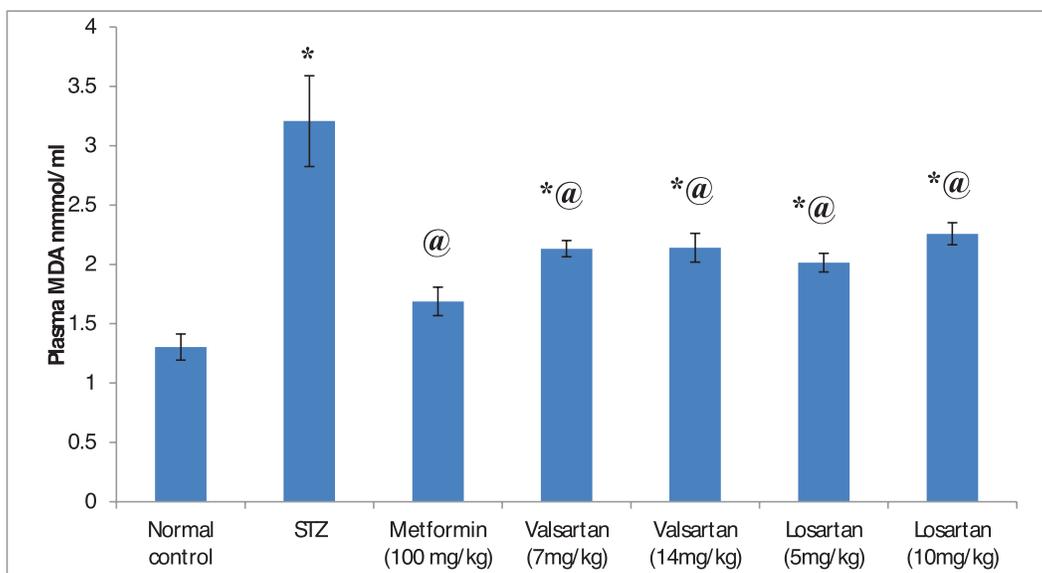


Fig. 3. Effects of valsartan (7 and 14 mg/kg, P.O.) and losartan (5 and 10 mg/kg, P.O.) in comparison with metformin (100 mg/kg/day, P.O.) on plasma MDA in STZ-induced diabetes in rats. Diabetes was induced in rats by single intraperitoneal injection of STZ. Valsartan, losartan, and metformin were orally administered daily for 6 weeks. Data are expressed as mean \pm SEM ($n = 6-8$). The statistical comparison of difference between groups was carried out using one-way ANOVA, followed by Duncan's multiple range test. *Statistically significant from normal control group at the corresponding time at $p < 0.05$. @Statistically significant from STZ control group at the corresponding time at $p < 0.05$.

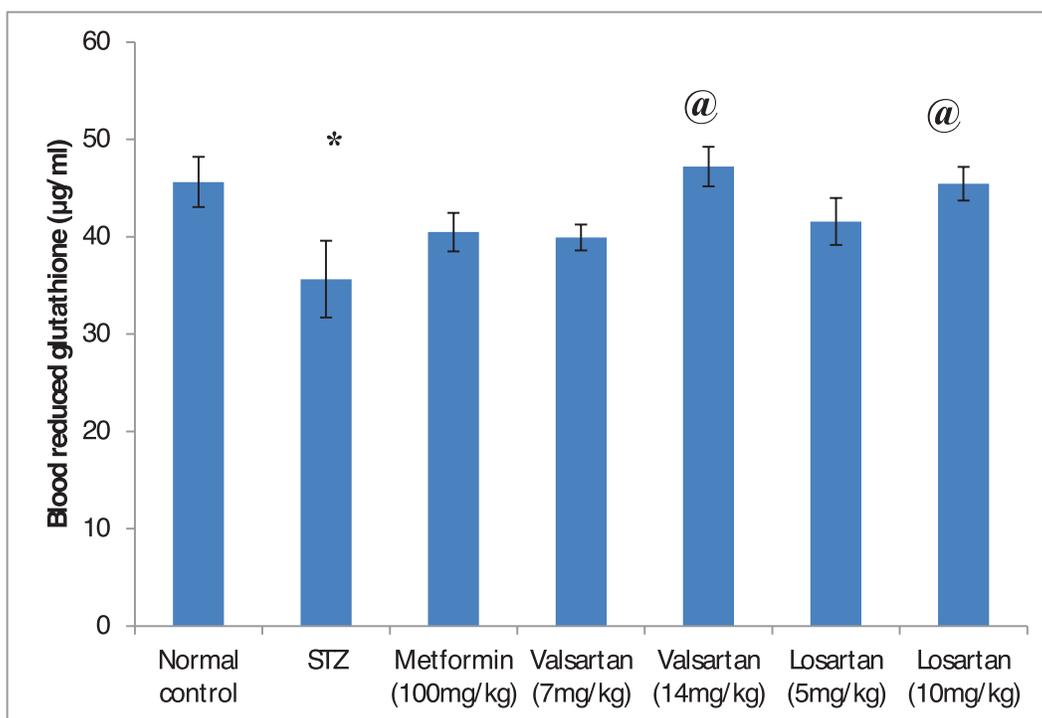


Fig. 4. Effects of valsartan (7 and 14 mg/kg, P.O.) and losartan (5 and 10 mg/kg, P.O.) in comparison with metformin (100 mg/kg/day, P.O.) on blood reduced glutathione (GSH) in STZ-induced diabetes in rats. Diabetes was induced in rats by single intraperitoneal injection of STZ. Valsartan, losartan, and metformin were orally administered daily for 6 weeks. Data are expressed as mean \pm SEM ($n = 6-8$). The statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA, followed by Duncan's multiple range test. *Statistically significant from normal control group at the corresponding time at $p < 0.05$. @Statistically significant from STZ control group at the corresponding time at $p < 0.05$.

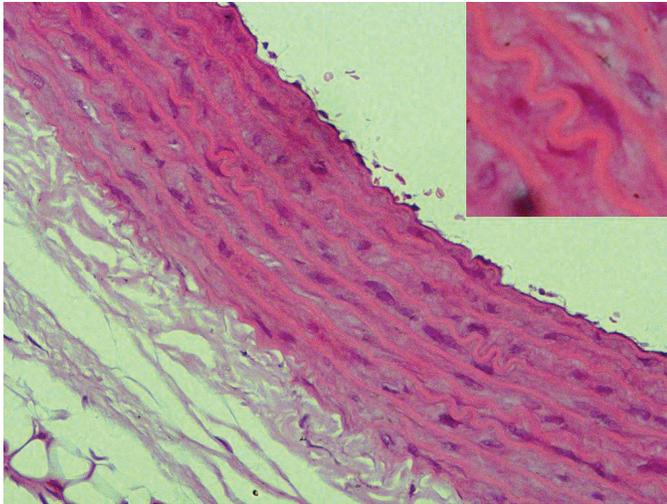


Fig. 5. A photomicrograph of an aortic section from a normal control rat showing intact tunica intima, thick tunica media occupying most of the thickness of the vessel wall with elastic fibers embedded in it and thin outer tunica adventitia made from loose connective tissue. The upper right corner of the figure shows the wavy elastic fibers characteristic of this vessel (H&E $\times 400$ & $1,000$).

Angiotensin II also has been shown to enhance superoxide generation through the activation of NAD(P)H oxidase independently of its systemic vasoconstriction (Fujita *et al.*, 2011); it stimulates the expression of leukocytes adhesion molecules and the release of inflammatory cytokines (Nasser *et al.*, 2014).

Furthermore, Kucharewicz *et al.* (2002) reported that Ang II could promote thrombosis through enhancing the secretion of plasminogen activator inhibitor type 1 (PAI-1) from endothelial and smooth muscle cells and increase tissue factor expression.

Additionally, the overexpression of the circulating and local levels of endothelin-1 (ET-1), as well as its abnormal signaling responses in diabetic animal models and diabetic patients has been discussed by Matsumoto *et al.* (2014). ET-1 is an endothelial cells (ECs)-derived peptide; it is a potent vasoconstrictor with mitogenic, pro-oxidative, and pro-inflammatory properties. The same authors also described the existing crosstalk between Ang II and ET-1. Ang II is able to enhance the expression of preproendothelin mRNA, and ET-1 in ECs, vascular smooth muscle cells (VSMCs), and vascular adventitial fibroblasts. In contrast, ET-1 was found to activate the conversion of Ang I to Ang II in pulmonary ECs. Moreover, the ET_A receptor antagonism was found to reduce the vasoconstrictor responses to Ang II.

Our results revealed that the induction of diabetes by the single i.p. injection of STZ resulted in a significant decrease in GSH level, as well as a significant elevation in plasma MDA level, suggesting increased generation of ROS, and these results were in agreement with other investigators (Sabry *et al.*, 2014; Singh *et al.*, 2016; Soon and Tan, 2002).

Excessive oxidative stress is a well-known pathogenic mechanism of diabetes and its micro- and macro-vascular complications. It arises from an imbalance between generation and elimination of ROS (Islam and Loots, 2007; Orhan *et al.*, 2011; Wang *et al.*, 2013). The increase in ROS generation has

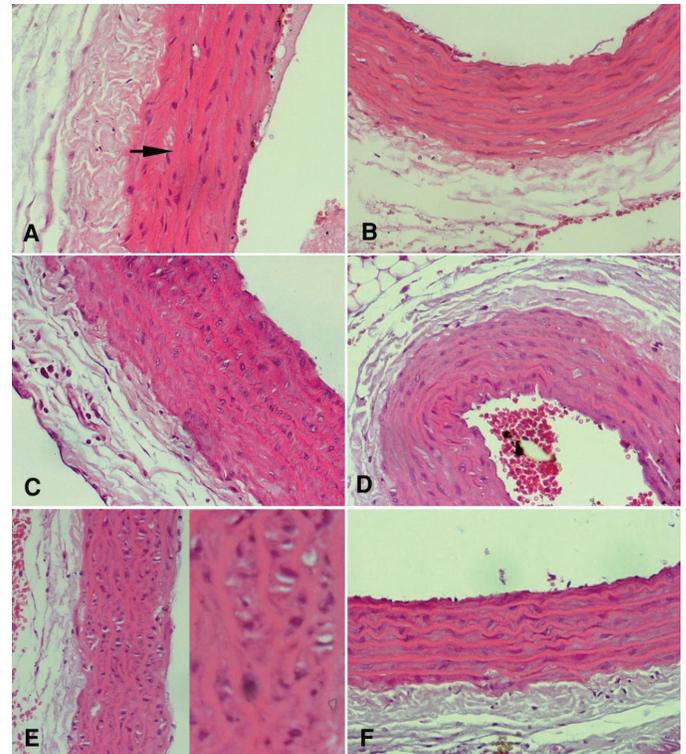


Fig. 6. A photomicrograph of aortic sections from: (A) Diabetic control rat showing thinning of the tunica media with stretching of the elastic fibers in it (arrow) with noticeable increase in thickness of adventitia. (B) Diabetic rat that received metformin 100 mg/kg/day showing regaining of the wavy elastic fibers in tunica media; however, the adventitia is still thicker than normal. (C) Diabetic rat that received valsartan 7 mg/kg/day showing wavy elastic fibers, thick adventitia, and abnormal-shaped muscle fibers of tunica media with swollen disoriented nuclei. (D) Diabetic rat that received valsartan 14 mg/kg/day showing normalization of the general architecture of the aortic wall, some nuclei of smooth muscle fibers are still swollen. (E) Diabetic rat that received losartan 5 mg/kg/day, the left half of the figure shows that the tunica media appeared thinner than normal with interlocking non-wavy elastic fibers. In the right half, the nuclei of smooth muscle fibers appeared swollen and disoriented. (F) Diabetic rat that received losartan 10 mg/kg/day showing normalization of the aortic wall (H&E $\times 400$).

been documented in both types of diabetes through multiple pathways such as increased lipid peroxidation, activation of protein kinase C (PKC), especially the β -isoform, activation of the NADPH oxidase, increased production of AGEs, redox imbalance secondary to enhanced aldose reductase (AR) activity, prostanoid imbalance, and mitochondrial overproduction of superoxide (Gupta *et al.*, 2010; Kellogg, 2008).

One of the mechanisms involved in increased ROS production during hyperglycemia is the diacylglycerol (DAG)—PKC pathway. The accumulation of DAG in chronic hyperglycemia contributes in the enhancement of PKC activity leading to excessive expression and activation of NADPH oxidase in all cell types within the vascular wall, hence the oxidative stress is increased (Gupta *et al.*, 2010).

Gupta *et al.* (2010) reported that the non-enzymatic glycosylation of protein leads to the formation of AGEs, and the AGEs alone and/or through its interaction with its receptor (RAGE) have the ability to further increase the oxidative stress.

They also have been shown to potentiate the NADPH oxidase activation resulting in superoxide production, which in turn leads to increased AGEs formation, creating a cyclical pattern with positive feedback.

Additionally, Koka *et al.* (2006) reported the ability of AGEs to stimulate the generation of angiotensin through the activation of chymase as an alternative pathway. This could underlie the greater therapeutic benefits of using ARBs when compared with the use of ACE inhibitors.

Another link between hyperglycemia and increased generation of ROS is the activation of the polyol pathway in response to the increase in the blood glucose concentration. The metabolism of glucose by the polyol pathway has been reported to contribute in the activation of AR2 and sorbitol dehydrogenase resulting in reduction of glucose to sorbitol and fructose. Activation of this pathway leads to increased NADPH consumption which is an essential cofactor for GSH generation (Gupta *et al.*, 2010; Thompson and Godin, 1995).

Furthermore, Gupta *et al.* (2010) and Musial *et al.* (2013) documented the ability of the increased superoxide generation to decrease nitric oxide (NO) bioavailability as a result of its chemical conversion to the potent oxidant peroxynitrite, resulting in decreased endothelial relaxation and elevated blood pressure.

The present histopathological examination of the aortic sections of diabetic rats showed several aortic alterations, including abnormal tunica media/adventitia ratio due to the thinning of the tunica media with stretching of the elastic fibers in it, and a noticeable increase in thickness of adventitia. This was in harmony with the results of Marquie *et al.* (1998). Patients suffering from DM are more exposed to the risk of atherothrombosis, which arises as a result of increased levels of several pro-coagulation factors (e.g., fibrinogen, factor VII, and von Willebrand factor) and a decrease in the levels of several anticoagulation factors (e.g., antithrombin III and protein C) (Ha *et al.*, 2014).

Hyperglycemia is a leading cause for glycosylation of proteins and phospholipids to form the chemically reversible early glycosylation products which subsequently undergo a complicated series of chemical rearrangements to produce the irreversible AGEs, which have the ability to induce cross-linking of extracellular matrix proteins leading to a decrease in the compliance and stiffening of the arterial wall. This could be clinically translated into systolic hypertension (Esper *et al.*, 2008; Gupta *et al.*, 2010; Sampathkumar *et al.*, 2005).

Regarding the present observed decrease in heart rate of the STZ- diabetic rats, similar results have been reported by several researchers (Goyal *et al.*, 2009; Hassan *et al.*, 2013; Meamar *et al.*, 2015). The developed bradycardia was thought to be attributed to alterations in the sinoatrial (SA) node and it was found to be associated with disease progression (Sanyal *et al.*, 2012; VanHoose, 2011).

Grise *et al.* (2016) reported that the STZ-treatment itself has the ability to lengthen the action potential duration in the SA node, thus slowing the heart rate; whereas, Howarth *et al.* (2005) documented the ability of insulin to reverse the reductions in heart rate observed in STZ-induced diabetic rats, suggesting that it is the diabetes rather than STZ treatment that is responsible for the bradycardia.

The current results demonstrated that the daily treatment of the STZ diabetic rats with valsartan (7 and 14 mg/kg) and losartan

(5 and 10 mg/kg) for 6 weeks resulted in the normalization of SBP without any significant change on the observed bradycardia.

Regarding the antihypertensive actions, the observed blood pressure lowering effect of valsartan and losartan at both dose levels was documented by several authors (Chan *et al.*, 2003; Hussien, 2014). Valsartan and losartan are safe and effective drugs belonging to the Angiotensin II, type 1 receptor blockers (ARBs) used for the treatment of hypertension (Hussien, 2014). Their main mechanism of action is attained through the inhibition of the Ang II signaling via blocking the AT1 receptors (Nasser *et al.*, 2014).

The unaffected bradycardia by valsartan and losartan was in harmony with the results of Fukui *et al.* (2009) who showed that administration of olmesartan which is another ARB resulted in a lower heart rate in diabetic hypertensive rats compared with the normal rats. On the other hand, Hussien (2014) reported that administration of losartan in STZ diabetic rats significantly increased the heart rate to reach the normal level. This discrepancy may be attributed to the duration of hyperglycemia and to the dose of the used drugs.

It has been reported that losartan significantly abolished the increase in blood pressure and inhibited the increase in aortic ET-1 content in Ang II-induced hypertension (d'Uscio *et al.*, 1998). Additionally, administration of losartan in a 2-weeks study, normalized diabetes-related impairments of insulin-induced relaxation in the aorta of Goto-Kakizaki (GK) type 2 diabetic rats (diabetic rats exhibiting insulin resistance and hyperinsulinemia). The effect of losartan on the insulin-induced aortic relaxation was endothelium dependent. This effect was attributed to the restoration of the NO production and eNOS phosphorylation, and the enhancement of insulin sensitivity (Nemoto *et al.*, 2011).

The present results also revealed that administration of valsartan and losartan succeeded in ameliorating the depleted GSH, as well as the elevated level of MDA observed in the STZ diabetic rats. This was in harmony with the results of Hussien (2014) and Senapaty *et al.* (2014).

Zhao *et al.* (2007) reported that valsartan treatment significantly enhanced the superoxide dismutase (SOD) activity and suppressed the levels of hydroxyl radical (OH[•]), MDA, and the expression of NADPH in the brain of diabetic rats.

In a similar pattern, systemic administration of losartan in diabetic hypertensive rats significantly reduced the expression of inducible nitric oxide synthase (iNOS) and reestablished the antioxidant system (Silva *et al.*, 2010). Evidences suggested that activation of iNOS causes DNA breaks with a subsequent activation of poly (ADP-ribose) polymerase (PARP) which is an important regulator of several pro-inflammatory mediators. It also has the ability to increase the leukocytes adhesion to the endothelial cells, as well as to enhance the formation of vascular endothelial growth factor (Silva *et al.*, 2010; Zakaria *et al.*, 2016).

Several studies demonstrated the ability of the ARBs to exert their cardiovascular protective actions through other mechanisms which are not just blockade of AngII signaling. Thomas *et al.* (2013) reported that valsartan treatment partially suppressed the elevated inflammatory cytokines, namely, TNF α , IL-1 β , IFN γ , and MCP-1 in STZ diabetic mice.

Similarly, valsartan through the activation of AMP-activated protein kinase succeeded in inhibiting the expression of early growth response factor 1 (Egr-1) in the aorta of STZ-induced diabetic mice. This response significantly resulted

in inhibiting the expression of the inflammatory cytokines, TLR-2 and TLR-4. Egr-1 is a nuclear protein that has been reported to act as a major contributor in the pathogenesis of atherosclerosis by inducing the formation of foam cells, with subsequent thrombosis and restenosis (Ha *et al.*, 2014).

In the current study, the histopathological examination of aortic sections from the valsartan and losartan treated diabetic rats at the high dose levels revealed regaining of the normal architecture of the aortic wall. These data were consistent with the results of Tikellis *et al.* (2013) who reported that blockade of the RAAS was able to inhibit the diabetes-associated atherosclerotic plaque formation in atherosclerosis-prone *apoE* KO (apolipoprotein E knockout)-mice, through blood pressure independent mechanism (Kurtz and Pravenec, 2008).

The beneficial effect of losartan administration in suppressing the progression of arterial stiffness when administered in hyperglycemic patients has been documented by Uehara and Takeda (2008). Also, Kurtz and Pravenec (2008) have pointed out the role of losartan and its metabolite EXP3174 in interfering with the platelet aggregation and vasoconstriction induced by a thromboxane receptor agonist, while valsartan appears to have little or no effect. They have discussed the ability of losartan to interfere with the binding of thromboxane A₂/prostaglandin endoperoxide H₂ (TxA₂/PGH₂) ligand to its receptors on platelets or coronary arteries, thereby promoting further protection against coronary events in a mechanism which is not just depend on AT1 receptor blockade.

The current results demonstrated that daily administration of metformin (100 mg/kg) to the STZ diabetic rats resulted in a numerical, but non-significant, tendency to decrease the blood pressure with no effect on heart rate.

However, Majithiya and Balaraman (2006) showed that administration of metformin resulted in a significant decrease in SBP.

Metformin is a biguanide oral antidiabetic agent used for the control of type 2 diabetes. Its hypoglycemic actions are mainly mediated through inhibition of gluconeogenesis, enhancement of hepatic insulin sensitivity, as well as stimulation of glucose uptake in skeletal muscle and adipocytes. Several authors have discussed the benefits of metformin in reducing the cardiovascular-related mortality rates in type 2 diabetic patients (Alhaider *et al.*, 2011; Ouslimani *et al.*, 2005; Rahimi *et al.*, 2005).

Majithiya and Balaraman (2006) discussed the beneficial effects of metformin in improving the vascular function in diabetic rats against the STZ- induced vasculopathy. They reported the ability of metformin to increase the activity of NO without affecting its expression. This effect was mediated through inhibiting the breakdown of NO by the activated ROS.

In the present piece of work, the daily metformin administration resulted in a significant decrease in the level of MDA associated with numerical non-significant increase in the blood GSH content when compared with the STZ diabetic untreated rats. In addition, the histopathological examination of the aortic sections from the metformin treated rats revealed almost regaining the normal architecture of the aortic wall.

The antioxidant properties of metformin have been documented by several authors (Sabry *et al.*, 2014; Singh *et al.*, 2016; Soon and Tan, 2002). They documented the upregulation

of the antioxidant enzymes such as SOD and catalase, as well as the increased GSH concentration in response to the administration of metformin. In addition, Rahimi *et al.* (2005) and Sabry *et al.* (2014) reported the ability of metformin to inhibit the generation of ROS by suppressing the activities of NAD(P)H oxidase and/or mitochondrial respiratory chain.

In summary, the present study showed that blockage of AT1 receptors with ARBs (valsartan and losartan) succeeded in ameliorating the cardiovascular hemodynamic abnormalities and the vascular structural aberrations associated with the STZ-induced diabetes. These protective effects could be in part mediated through ameliorating the generation of ROS.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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