IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
January 2018 Vol.:11, Issue:2

The Protective Effect of Renin-Angiotensin-Aldosterone System Inhibitors in STZ- Induced Diabetes Mellitus in Rats

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Hassan Helaly Abu Rahma, Ali Abdel Salam A. Attia*, Sherif Motawie Abdel Fadheel

Department of pharmacology, Faculty of Medicine, Al-Azhar University (Assiut)

Submission: 30 December 2017
Accepted: 5 January 2018
Published: 30 January 2018

www.ijppr.humanjournals.com

Keywords: Protective Effect, Renin-Angiotensin-Aldosterone System, Diabetes Mellitus, Rats

ABSTRACT

Diabetes is a serious metabolic disorder with micro and macrovascular complications that result in significant morbidity and mortality. Hyperglycemia induces endothelial dysfunction through the generation of oxidative stress which has been suggested to be the key player in the generation of renal and cardiovascular complications. The present study aimed to assess the possible protective role of Renin-Angiotensin-Aldosterone System (RAAS) blockers in the DM-induced vascular and biochemical changes in the STZ experimentally induced diabetes mellitus in rats. The antidiabetic drug, Gliclazide (10mg/kg), the angiotensin antagonist, telmisartan (5mg/kg), The ACE inhibitor, lisinopril (10mg/kg) and the aldosterone antagonist, spironolactone (50mg/kg) are given daily orally for 8 weeks. After 8 weeks of treatment, blood samples were withdrawn for measurement of fasting blood glucose level, serum insulin level, and antioxidant parameters. The reactivity of the isolated rat's aortae to norepinephrine and acetylcholine was also measured. The results show that RAAS blockers (telmisartan, lisinopril, and spironolactone) decreased the enhanced aortic contractility and increased the acetylcholine-induced relaxations of the rat aorta as compared with those obtained from the diabetic non treated rats. Telmisartan, unlike other RAAS blockers, has the ability to decrease blood glucose and increase serum insulin levels in the diabetic rats. Telmisartan synergizes the hypoglycemic effect of gliclazide. Serum Superoxide Dismutase (SOD) and glutathione (GSH) levels were increased significantly in the diabetic rats treated with gliclazide. RAAS blockers increase significantly the GSH & SOD levels and enhance the activity of gliclazide. Malondialdehyde (MDA), is increased in the diabetic rats. RAAS blockers decrease significantly the serum levels of MDA. In conclusion, inhibitors of the Renin Angiotensin Aldosterone system can enhance significantly the antioxidant activity of gliclazide and protect against diabetes-induced cardiovascular complications.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both **(Maiese et al., 2007).** Diabetes is a serious disorder with micro and macrovascular complications that result in significant morbidity and mortality **(Grundy et al,. 1999).** Several studies have shown that hyperglycemia induces endothelial dysfunction through the generation of oxidative stress which has been suggested to be the key player in the generation of cardiovascular complications **(Brownlee, 2001).** Gliclazide is a sulphonylurea drug and used in the treatment of type II diabetes. It is generally well tolerated, associated with a relatively low incidence of hypoglycemia and may have beneficial effects beyond the reduction of serum glucose **(De Mattia et al., 2003).** Gliclazide effectively maintains glycemic control possibly by pancreatic and extrapancreatic effects **(Bak and Pedersen 1991).** The renin-angiotensin system (RAS) plays a crucial role in circulatory homeostasis and the regulation of vascular tone. There is a growing body of evidence that enhanced activation of RAS and the subsequent increase of angiotensin II & aldosterone levels contribute to changes of the insulin/ IGF-1 signaling pathway and promote the formation of ROS (reactive oxygen species) that induces endothelial dysfunction & CVD. Therefore, both hyperglycemia and angiotensin II-mediated action lead to oxidative stress and play a central role in the progression of diabetes and development of a diabetic complication**.** Angiotensin II, type 1 receptor blockers (ARBs) are safe and effective drugs for the treatment of hypertension. Exogenous administration of angiotensin II receptor antagonist may be beneficial in counteracting functional changes of atherosclerosis because the rennin-angiotensin system has been reported to be an important contributory factor in the pathophysiology of CVD **(Cooper et al., 2007).**

AIM OF THE WORK

The aim of the present study is to evaluate the role of the renin-angiotensin-aldosterone system in diabetes mellitus by studying and comparing effects of inhibitors of the RAAS; angiotensin receptor blocker (telmisartan), angiotensin-converting enzyme inhibitor (lisinopril) and aldosterone antagonist (spironolactone) on the blood glucose level, insulin level, oxidative stress parameters and the aortic reactivity to both the contractile agent, noradrenaline and the endothelial-dependent vasodilator acetylcholine in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Adult male albino rats with initial body weights ranging from 150 to 200g are chosen as an animal model for this study. Diabetes mellitus was induced in rats by single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body STZ induces diabetes within 3 days by destroying the beta cells **(Chatzigeorgiou et al., 2009),** The rats were divided into nine groups (8 rats each) and treated for 8 weeks as follow: The first group is served as control (nondiabetic), the second is served as diabetic rats treated with distilled water. Other groups are diabetic rats treated by single dose orally daily with gliclazide (10mg/kg) **(Abd Ellraheim et al., 2015),** telmisartan (5mg/kg) (**Snigdha et al., 2014)**, gliclazide (10mg/kg) and telmisartan (5mg/kg), lisinopril (10 mg/kg) **(Abd Ellraheim et al., 2015),** gliclazide (10mg/kg) with lisinopril (10 mg/kg), spironolactone (50 mg/ kg) **(Banki et al., 2012),** gliclazide (10mg/kg) with spironolactone (50 mg/ kg) respectively**.** At the end of the experimental period, which lasts for 8 weeks, blood samples were collected. Blood glucose concentration, blood insulin level, lipid peroxidation, superoxide dismutase, and glutathione were assayed after the withdrawal of the blood samples. The animals were sacrificed and the aortae were isolated to investigate its responsiveness to the vasoconstrictor (norepinephrine) and the vasodilator (acetylcholine) agents. The results are expressed as mean +/- s.e.mean. For the comparison of statistical significance, Student's t-test was used. P values of less than 0.05 were considered significant.

RESULTS

Cumulative concentration-response curves were obtained for noradrenaline on the rat aortae. The contractile responses of the rat aortae of the diabetic rats to noradrenaline were increased significantly ($P< 0.001$) as compared with the responses obtained from the nondiabetic rats. Treatment with the antidiabetic drug, gliclazide decreased significantly ($P< 0.01$) the contractile responses of rat aortae to noradrenaline as compared with those of the diabetic non-treated rats. Treatment with the angiotensin antagonist, telmisartan, the ACE inhibitor, lisinopril and the aldosterone antagonist spironolactone separately decreased significantly ($P< 0.01$) the contractile responses of rat aortae to noradrenaline as compared with those of the diabetic non-treated rats. Treatment with a combination of telmisartan & gliclazide produced marked significant decreased ($P< 0.001$) on the contractile response of the aortae of the diabetic rats to noradrenaline. Treatment with the combination of gliclazide with either

lisinopril or spironolactone produced the significant decrease of the contractile responses of rat aortae to noradrenaline (P< 0.01) as shown in figures (1&2).

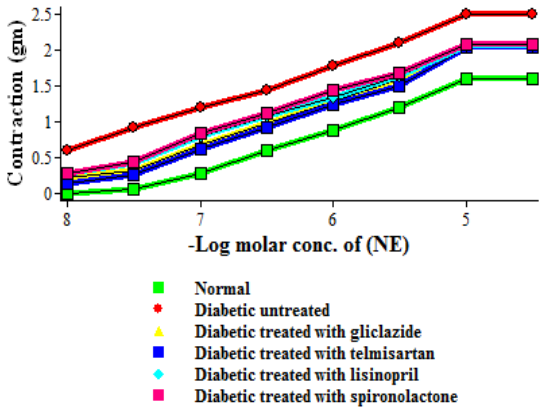

Figure (1): Effect of pretreatment with gliclazide, telmisartan, lisinopril and spironolactone on the contractile response of the diabetic rat's isolated aortae to norepinephrine

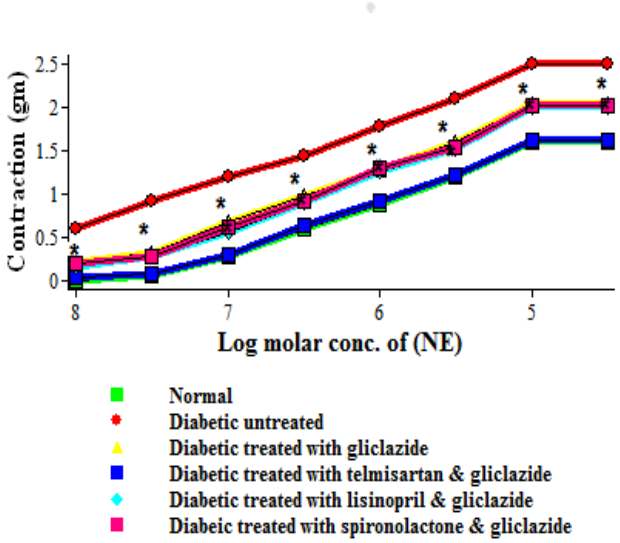

Figure (2): Effect of pretreatment with gliclazide alone and the combination of gliclazide with telmisartan, lisinopril, and spironolactone on the contractile response of the diabetic rat's isolated aortae to norepinephrine.

Each value represents the mean of 7 – 9 rats.

* Significant difference from diabetic rats treated with telmisartan & gliclazide (P< 0.01).

Cumulative concentration-response curves of acetylcholine were obtained on the noradrenaline precontracted rat aortae. The relaxant responses of the rat aortae of the diabetic rats to acetylcholine were decreased significantly (P< 0.01) as compared with the responses obtained from the nondiabetic rats. Treatment with the antidiabetic drug, gliclazide increased significantly (P< 0.01) the relaxant responses of rat aortae to acetylcholine as compared with those of the diabetic non-treated rats**.** Treatment with the angiotensin antagonist, telmisartan, the ACE inhibitor, lisinopril and the aldosterone antagonist spironolactone separately increased significantly (P< 0.01) the relaxant responses of rat aortae to acetylcholine as compared with those of the diabetic non-treated rats. Treatment with a combination of telmisartan & gliclazide produced significant increase (P< 0.01) on the relaxant response of the aortae of the diabetic rats to acetylcholine. Treatment with the combination of gliclazide with either lisinopril or spironolactone produced a significant increase of the relaxant responses of rat aortae to acetylcholine (P< 0.01) as shown in figure 3&4.

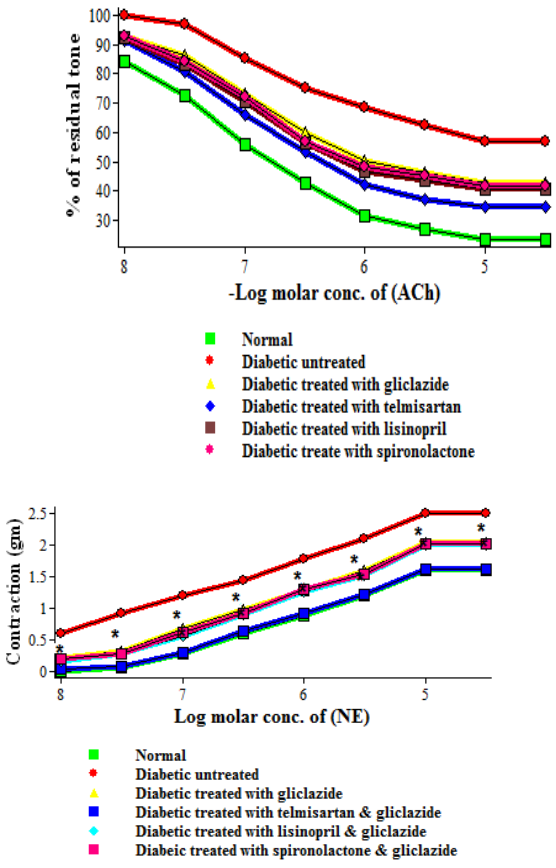

Figure (3): Effects of pretreatment with gliclazide, telmisartan, lisinopril and spironolactone on the relaxant response of the diabetic rat's isolated aortae to acetylcholine.

Each value represents mean of 7 – 9 rats.

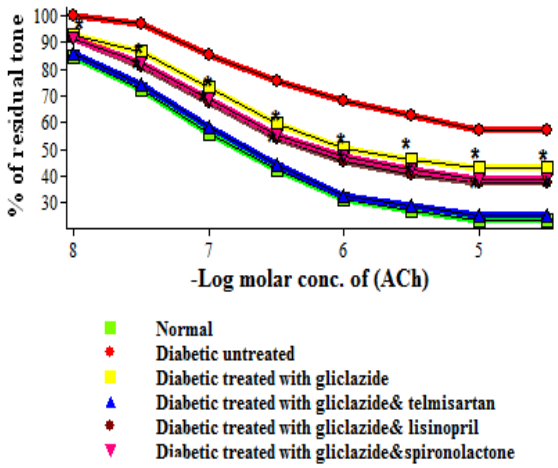

Figure (4): Effect of pretreatment with gliclazide alone and the combination of gliclazide with telmisartan, lisinopril or spironolactone on the relaxant response of the diabetic rat's isolated aortae to acetylcholine.

* Significant difference from diabetic rats treated with telmisartan & gliclazide (P< 0.01)

Fasting blood glucose was decreased significantly (P< 0.01) In the diabetic rats treated with gliclazide or telmisartan. Treatment with the combination of telmisartan and gliclazide significantly decreased (P< 0.001) the fasting blood glucose of the diabetic rats. Treatment with lisinopril or spironolactone did not affect the fasting blood glucose of the diabetic rats. However, treatment with combination of gliclazide with either Lisinopril or spironolactone significantly decreased (P< 0.01) the fasting blood glucose of the diabetic rats as shown in figure -5 & 6.

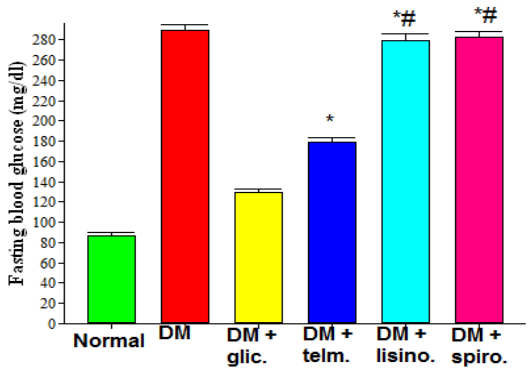

Figure 5: Effects of pretreatment with gliclazide, telmisartan, lisinopril and spironolactone on fasting blood glucose level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

* Significant difference from the diabetic rats treated with gliclazide (P<0.01).

Significant difference from the diabetic rats treated with telmisartan (P<0.01).

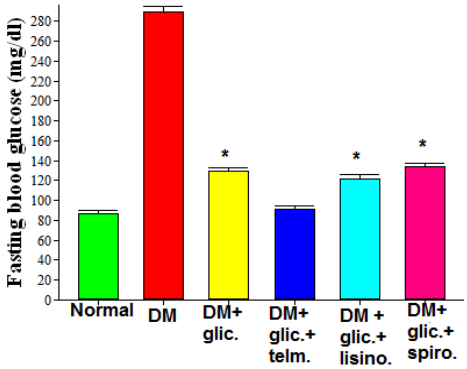

Figure (6): Effects of pretreatment with gliclazide alone and a combination of gliclazide with telmisartan, lisinopril, and spironolactone on fasting blood glucose level of the diabetic rats.

Each value represents the mean SE (standard error) of 7 – 9 animals.

* Significant difference from the diabetic rats treated with telmisartan & gliclazide (P<0.01).

Serum insulin level is decreased significantly in the diabetic non treated rats. Serum insulin was increased significantly (P< 0.01) In the diabetic rats treated with either gliclazide or telmisartan. Treatment with the combination of telmisartan & gliclazide significantly increased (P< 0.001) the serum insulin level of the diabetic rats. The serum insulin level of the diabetic rats treated with either Lisinopril or spironolactone did not significantly affected. Treatment with the combination of lisinopril & gliclazide significantly decreased (P< 0.01) the serum insulin level of the diabetic rats as shown in figure 7& 8.

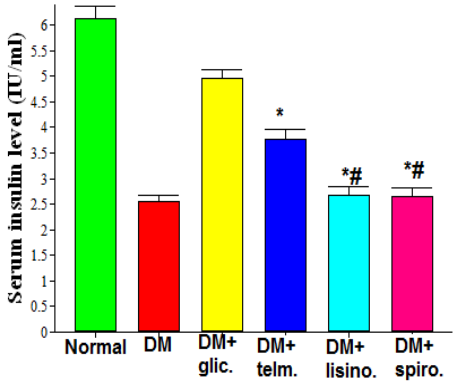

Figure (7): Effects of pretreatment with gliclazide, telmisartan, lisinopril and spironolactone on serum insulin level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

* Significant difference from the diabetic rats treated with gliclazide (P<0.01).

Significant difference from the diabetic rats treated with telmisartan (P<0.01).

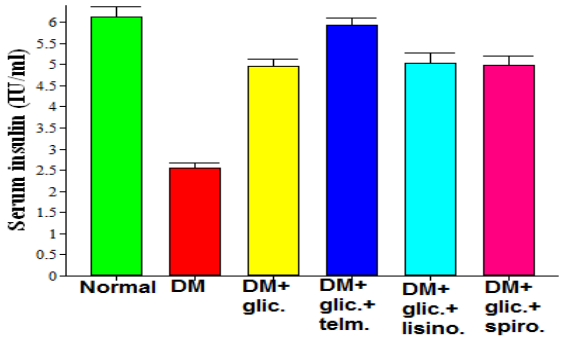

Figure (8): Effects of pretreatment with gliclazide alone and combination of gliclazide with telmisartan, lisinopril, and spironolactone on serum insulin level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

Serum Superoxide Dismutase (SOD) and glutathione (GSH) levels were decreased in the diabetic rats and increased significantly (P< 0.01) with treatment by gliclazide. Serum SOD and GSH levels of the diabetic rats treated with telmisartan were increased significantly (P< 0.01). Treatment with a combination of telmisartan & gliclazide significantly increased (P< 0.001) serum SOD and GSH levels of the diabetic rats. Serum SOD and GSH levels of the diabetic rats treated with lisinopril was increased significantly (P< 0.01). Treatment with a

combination of lisinopril & gliclazide significantly increased (P< 0.001) serum SOD and GSH levels of the diabetic rats. Serum SOD and GSH levels of the diabetic rats treated with spironolactone was increased significantly (P< 0.01). Treatment with a combination of spironolactone & gliclazide significantly increased (P< 0.001) SOD and GSH levels of the diabetic rats. Serum malondialdehyde (MDA) was increased in the diabetic rats and decreased significantly (P< 0.01) in the diabetic rats treated with gliclazide. Serum MDA in the diabetic rats treated with telmisartan was decreased significantly (P< 0.01). Treatment with a combination of telmisartan & gliclazide significantly decreased (P< 0.001) Serum MDA in the diabetic rats. Serum MDA in the diabetic rats treated with lisinopril was decreased significantly (P< 0.01). Treatment with a combination of lisinopril & gliclazide significantly decreased (P< 0.001) serum MDA in the diabetic rats. Serum MDA in the diabetic rats treated with spironolactone was decreased significantly (P< 0.01). Treatment with a combination of spironolactone & gliclazide significantly decreased (P< 0.001) serum MDA in the diabetic rats AS shown in figures 9-14.

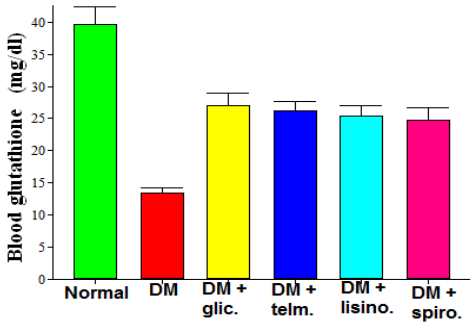

Figure (9): Effects of pretreatment with gliclazide, telmisartan, lisinopril and spironolactone on blood glutathione level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

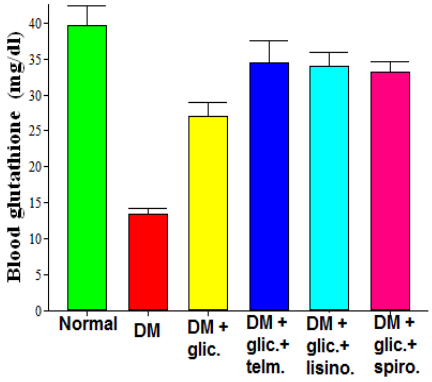

Figure (10): Effects of pretreatment with gliclazide alone and combination of gliclazide with telmisartan, lisinopril or spironolactone on serum reduced glutathione of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

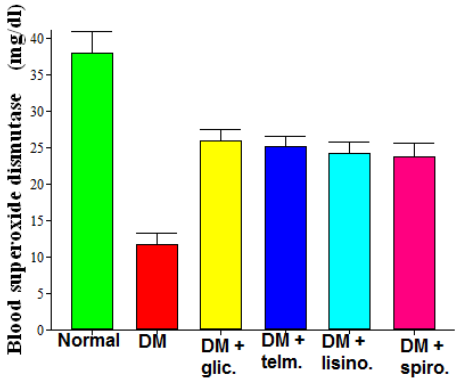

Figure (11): Effects of pretreatment with gliclazide, telmisartan, lisinopril and spironolactone on blood superoxide dismutase level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

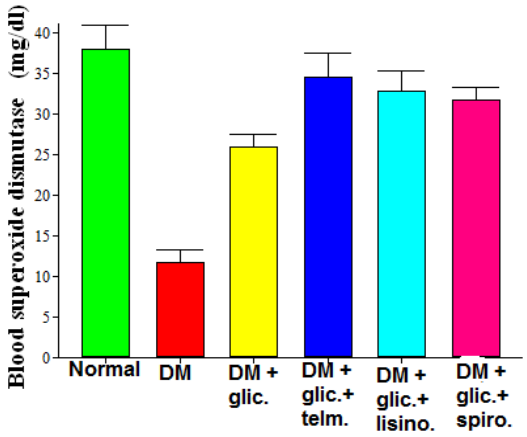

Figure (12): Effects of pretreatment with gliclazide alone and combination of gliclazide with telmisartan, lisinopril or spironolactone on blood superoxide dismutase level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

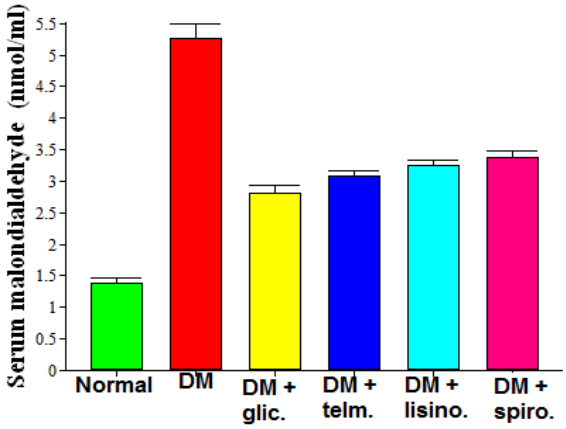

Figure (13): Comparison between effects of pretreatment with gliclazide, telmisartan, lisinopril and spironolactone on serum malondialdehyde level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

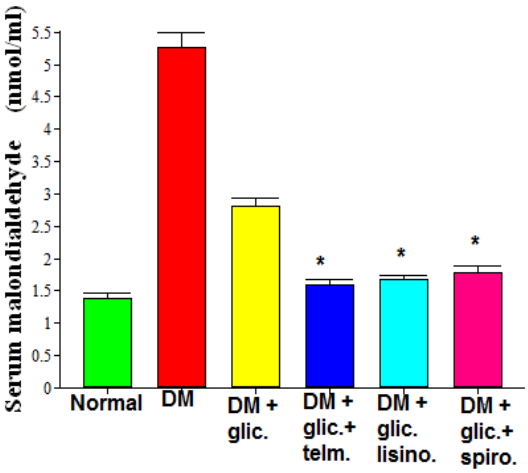

Figure (14): Effects of pretreatment with gliclazide alone and combination of gliclazide with telmisartan, lisinopril or spironolactone on serum malondialdehyde level of the diabetic rats

Each value represents the mean SE (standard error) of 7 – 9 animals.

DISCUSSION

Diabetes is a serious disorder with micro and macrovascular complications that result in significant morbidity and mortality. The incidence of CVD in diabetic patients has increased up to 3 folds and is a leading cause of death worldwide **(Grundy et al,. 1999).** Several studies have shown that hyperglycemia induces endothelial dysfunction through the generation of oxidative stress, which has been suggested to be the key player in the generation of cardiovascular complications **(Brownlee, 2001).** The renin-angiotensin system plays a crucial role in circulatory homeostasis and the regulation of vascular tone. There is growing body of evidence that enhanced activation of RAAS and the subsequent increase of AII & aldosterone levels contribute to changes of the insulin-signaling pathway and promote the formation of ROS that induces endothelial dysfunction & CVD**.** Therefore, both hyperglycemia and AII mediated action lead to oxidative stress and play a central role in the progression of diabetes and development of diabetic complication. Renin-angiotensin system inhibitors are safe and effective drugs for the treatment of hypertension. Exogenous administration of RAAS blockers may be beneficial in counteracting functional changes of atherosclerosis because the RAAS has been reported to be an important contributory factor in the pathophysiology of CVD **(Cooper et al., 2007).** The present work aimed to study and evaluate the effects of RAAS inhibitors; ACE inhibitor (lisinopril), ARB (telmisartan) and

aldosterone antagonist (spironolactone) on the vascular reactivity of the diabetic rat's aortae and the DM-induced biochemical changes. The results of the present study showed that the contractile response of the rat's isolated aortae induced by norepinephrine (NE) is increased significantly in the diabetic untreated rats in comparison with the normal rats and the relaxant response to acetylcholine (ACh) on NE precontracted aortic ring preparations was decreased significantly in the diabetic untreated rats in comparison with the normal rats. The results obtained by the present study are in agreement with the results obtained by other studies; **Desoky et. Al., 2014**, **Xavier et. Al., 2003**, **Mohd & Macha 2006**, **Roghani et. Al., 2013 ,Pagano et. al., 1998**, **Ramanadham et, al., 1984**, **Chang and Stevens 1992** , **Heygate et. al., 1995.** However**, Xavier et. Al., 2003,** reported that there was no significant difference in the maximum contractile response or sensitivity to noradrenaline in either of the diabetic groups compared to their age-matched controls. Reasons for these differences are not clear, although they could be explained by different experimental conditions including the type of artery, the type of agonist examined, the method used for vascular studies or the duration of diabetes. The results of the present study show that the contractile response of the aorta induced by NE is decreased significantly in the diabetic rats treated with gliclazide in comparison with the diabetic untreated rats. In addition, the relaxant response of Ach on NE precontracted aortic ring preparations was increased significantly in the diabetic rats treated with gliclazide in comparison with the diabetic untreated rats. The obtained results from the present study are in agreement with the results obtained by other workers; **Sena et. al., 2009** , **Vallejo et. al., 2000.**. The results of the present study show that the contractile response of the aorta induced by NE is decreased significantly in the diabetic rats treated with telmisartan in comparison with the diabetic untreated rats. Also, the relaxant response of Ach on NE precontracted aortic ring preparations was increased significantly in the diabetic rats treated with telmisartan in comparison with the diabetic untreated rats, but still there was a significant difference in comparison with the normal rats, while treatment with a combination of telmisartan & gliclazide normalize the endothelial functions of the aortae of the diabetic rats. The obtained results from the present study are in agreement with the results obtained by other studies; **Mollnau et. al., 2013**& **Schäfer et. al., 2007**. **Silva et. al., 2015** reported that spironolactone decreases diabetes-associated vascular oxidative stress and prevents vascular dysfunction (improve endothelium-dependent vasodilation, represented by increased Ach-induced relaxation), through processes involving increased expression of antioxidant enzymes. The present work show that fasting blood glucose was increased significantly and serum insulin level was decreased significantly in the diabetic untreated rats in comparison

with the normal rats. In the diabetic rats treated with gliclazide, fasting blood glucose was decreased significantly and serum insulin level was increased significantly in comparison with the diabetic untreated rats, and this finding was in agreement with the results obtained by **Abd El Motteleb and Abd El Aleem 2017 & Abd Ebrahim et al., 2015**. These findings are in agreement with the results obtained by other studies; **Khan and Gupta 2015**, **Goyal et al., 2011a, Senapaty et al., 2014**. **Benson et al., 2004** found that the Ang II receptor antagonist telmisartan is also a partial agonist of PPAR, a well-known target of insulin-sensitizing drugs used to treat type 2 diabetes. In contrast, none of the other ARBs affected PPAR activity. The mechanism whereby telmisartan activates PPAR remains to be determined; however, given the substantial chemical structural differences between telmisartan and all of the other commercially available ARBs, it is not surprising that telmisartan has unique biologic properties. There are a number of possible mechanisms that could theoretically mediate the effects of telmisartan on PPAR activity, including but not limited to effects on the conformation or phosphorylation status of the receptor, effects on the activity of coactivators or corepressors that modulate the transcriptional effects of PPAR, or even effects on endogenous ligands of PPAR**.** The results of the present study are also supported by the results obtained by **Hamed and Malek 2007** who showed that telmisartan therapy resulted in a significant decrease in serum glucose and HBA1c levels in the diabetic rats that may be considered an improvement in glycemic status. Telmisartan has the ability to activate PPAR involved in carbohydrate and lipid metabolism, beside its ability to regulate blood pressure and oxidative stress production. The results of the present study show that treatment with lisinopril or spironolactone has no significant effect on blood glucose or serum insulin levels. These results are in agreement with other workers; **Kojima et al., 2015**,. **Paliwal et al., 2014**,. **Arora and Singh 2013**, Silva **et al., 2015 & Goyal et al., 2011b** & **Mayyas et al., 2017.** The results of the present study were in disagreement with the results obtained by other workers; **Agrawal and Gupta 2013** mention that lisinopril has showed significant anti-hyperglycemic effect in diabetic rats and it enhanced the hypoglycemic activity of oral anti-diabetic drugs (Metformin, Gliclazide and Pioglitazone) in highly significant manner. They added that the present findings suggest that lisinopril may have some insulin sensitivity potentiating properties in T2DM but not in normoglycemic rats. Though the mechanism for this drug-induced hypoglycaemia is not well defined, it is proposed that the increase in bradykinins associated with ACE inhibitor use may cause an increase in insulin sensitivity**. Banki et al., 2012** reported that spironolactone reduced the elevated blood glucose level of diabetic animals. Although STZ injection leads to the

destruction of pancreatic ß-cells, a residual insulin activity still exists even after 6 weeks. Since aldosterone impairs insulin signaling, it is conceivable that spironolactone and eplerenone might be effective through inhibiting aldosterone induced insulin resistance. The differences observed in these results may be due to the difference in the dose and duration of drug treatment schedule or severity of hyperglycaemia. It has been reported that in STZ-diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia. A low level of plasma HDL is one component of a cluster of coronary disease risk factors that also includes abdominal obesity, hypertension and insulin resistance **(Goyal et al., 2011a).** Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defense mechanisms. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. The increase in the level of ROS in diabetes could be due to their increased production and/ or decreased destruction by nonenzymic and enzymic CAT, GSH-Px, and SOD antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. In addition, this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses **(Robertson, 2004).** Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation, which is responsible for increased incidence of atherosclerosis, a major complication of DM. An enhanced oxidative stress has been observed in these patients as indicated by increased free radical production, lipid peroxidation and diminished antioxidant status **(Moussa, 2015).** Free radicals may play an important role in the causation and complications of DM. Oxidative stress is currently suggested as a mechanism underlying diabetes and diabetic complication. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental DM, are thought to be the etiology of chronic diabetic complications. In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications **(Moussa, 2015).** The results of the present study showed that blood level of glutathione and superoxide dismutase was decreased significantly, while serum malondialdehyde was increased significantly in the diabetic untreated rats as compared with the normal rats. Treatment with gliclazide significantly increased the reduced levels of glutathione and superoxide dismutase and decreased the elevated levels of serum malondialdehyde in comparison with the diabetic untreated rats. These results are in

agreement with the results obtained by other workers; **Abd Ellraheim et al., 2015** show that STZ (50 mg/kg) significantly decreased blood GSH, serum NO and blood SOD levels and significantly increased serum MDA level as compared to normal control value. Treatment with gliclazide significantly reduced serum MDA level and increased blood GSH level, blood SOD activity and serum NO level of diabetic rats. Gliclazide possibly exerts such antioxidant effects due to the characteristics of its molecular structure. **Abd El Motteleb and Abd El Aleem 2017** reported that there was a significant increase in MDA (marker of lipid peroxidation) in the diabetic untreated group; gliclazide caused a significant reduction of elevated MDA. There was a significant reduction in GSH in the diabetic untreated group as compared to the control group, administration of gliclazide caused significant elevations of the reduced values of GSH. SOD showed significant reduction in the diabetic untreated group as compared to the control value, administration of gliclazide caused significant elevations of the reduced values of SOD **Hamed and Abdel Malek 2007** reported that diabetes produced a significant decrease in tissue GSH in liver, kidney and urinary bladder as compared to control group. In addition, diabetes produced a significant decrease in tissue SOD in liver, kidney and urinary bladder as compared to control group, while there was a significant increase in tissue lipid peroxides in liver, kidney and urinary bladder in the diabetic group as compared to control group. Gliclazide produced a significant decrease in lipid peroxides and concomitant increase in tissue GSH and SOD activity in diabetic rats. This could be explained by the reduction of hyperglycemia which stimulates the production of superoxide anion formation that interact to form a strong oxidant "peroxynitrite" which attacks various biomolecules in the vascular endothelium, vascular smooth muscle, myocardium and leads to development of diabetic nephropathy, retinopathy and neuropathy. The results of the present study showed that treatment of the diabetic rats with either telmisartan, lisinopril or spironolactone significantly increased the reduced levels of glutathione and superoxide dismutase and decreased the elevated levels of serum malondialdehyde in comparison with the diabetic untreated rats. Telmisartan is more effective in improving antioxidant status than lisinopril and spironolactone but these differences are not significant statistically. These results are in agreement with the results obtained by other studies; **Senapaty et al. 2014** reported that there was a significant increase in tissue MDA level in liver and kidney in diabetic group compared to control group. Administration of telmisartan significantly decreased MDA level in diabetic rats. Losartan and valsartan treated groups also produced a significant decrease in diabetic rats. The effect of telmisartan on tissue MDA levels was significantly greater when compared to the effect of losartan and valsartan. Hepatic & renal

GSH and SOD activities significantly decreased in Diabetic Control when compared with normal control. Administration of telmisartan, Losartan and valsartan exhibited significant increase in the levels of GSH & SOD in diabetic rats. However, the increase in the levels of GSH & SOD produced by telmisartan treated diabetic rats was significantly higher when compared to losartan and valsartan treated diabetic rats (**Senapaty et al. 2014)**. Experimental studies in both animals and humans have demonstrated that the ACE inhibitors and ARBs possess antioxidant effects through their action on the AT1R and AT2R **(Watanabe et al. 2005).** However, **Senapaty et al. 2014** suggest that telmisartan was more effective than losartan & valsartan in reducing the oxidative stress. There is a correlation between the decrease in hyperglycemia and the reduction of oxidative stress. Telmisartan has the capacity to both activate PPARs and block angiotensin receptors. Because such compounds exert anti-inflammatory effects through multiple pathways (PPAR pathway and the angiotensin receptor pathway), they provide a superior ability to treat or prevent inflammatory diseases than PPAR activators or ARBs alone. Such compounds that activate PPARs and block angiotensin receptors are also superior to PPAR activators because unlike currently recognized PPAR activators, the compounds of the current invention do not promote or aggravate fluid retention, peripheral edema, pulmonary edema or congestive heart failure. Therefore, it was likely that telmisartan alleviated the lipid peroxidation and tissue injuries through antihyperglycemic and antioxidant enzyme activity **(Senapaty et al. 2014). Hamed and Abdel Malek 2007** reported that Telmisartan administration in diabetic rats produced a significant decrease in lipid peroxides that were increased in long-duration diabetes in blood, heart, pancreas and urinary system. The significant decrease in lipid peroxides could be due to a decrease in oxidative stress induced by hyperglycemia under telmisartan effect that blocked angiotensin II action at its receptors. On the other hand, telmisartan administration produced a significant elevation of tissue GSH and SOD in diabetic rats. Blockade of angiotensin action could ameliorate oxidative stress induced by angiotensin that generates free radicals resulted in oxidative stress that consume GSH, furthermore, increase GSH biosynthesis may be a direct effect of telmisartan (angiotensin blocker).

The results of the present study are also in line with the results of the clinical study by **Hassan and Al-Hammami 2012** who showed that type 2 diabetic hypertensive patients revealed a significant rise in serum MDA and total antioxidants status (TAS) at baseline stage, however, one month monotherapy with either lisinopril or telmisartan results in a significant improvement in both above parameters. They added that ACEIs and ARBs may

act as ‘‘magic bullets" against oxidative stress and this may explain some of their beneficial effects that cannot be associated with their action on blood pressure. ACEIs and ARBs may affect ROS by their antioxidant effect through the free radical scavenger action or inhibition of molecules adhesion. They could also modulate ROS and RNS generation by inhibiting the stimulation of vascular *NADPH oxidase;* another predictable effect of ACEIs would be to reduce the ambient levels of superoxide in the vascular wall. They concluded that both lisinopril and telmisartan have a good efficacy against oxidative stress with no significant differences between them. **Polizio and Pena, 2007** showed that Lisinopril inhibits lipid peroxidation elevated tissue glutathione levels, and influenced the activity of antioxidant enzymes such as CAT and GPx. **Waanders et al., 2008** found that lisinopril, spironolactone and the combined treatment all tended to reduce urinary MDA levels compared to vehicle, without differences between the groups. **Yuan et al., 2007** show that significant increase in MDA levels and the decrease in SOD and GSH activities were found in the diabetic model group when compared with the control group. The treatment with spironolactone decreased MDA levels and increased SOD and GSH activities. Spironolactone can function as an antioxidant factor and protect organs from oxidative damage by enhancing antioxidative defense systems and suppressing production of free radicals.

REFERENCES

1. **Abd El Motteleb DM. and Abd El Aleem DI. (2017):** Renoprotective effect of Hypericum perforatum against diabetic nephropathy in rats: Insights in the underlying mechanisms. Clin Exp Pharmacol Physiol. 44:509–521.
2. **Abd El raheim M. A., Amira M. Abo-Youssef, Hany A. Omar and Hekma A. Abd El-Latif (2015):.** 'Angiotensin Inhibitors Potentiate the Hypoglycemic and Antioxidant Effects of Gliclazide in Rats.' Int. J. Pharm. Sci. Rev. Res., 31(1), Article No. 16, Pages: 75-80.
3. **Agrawal N. K. and Gupta U. (2013):** The effect of Lisinopril on blood glucose level given alone and in combination with oral antidiabetic drugs in alloxan-induced diabetic rats. Afr. J. Pharmacol. Ther. 2(2): 59-65.
4. **Arora M. K. and Singh U. K. (2013):** Combination of PPAR-α Agonist and DPP-4 Inhibitor: A Novel Therapeutic Approach in the Management of Diabetic Nephropathy J Diabetes Metab, 4:10
5. **Bak JF and Pedersen O, (1991):** 'Gliclazide and insulin action in human muscle.' Diabetes Research and Clinical Practice, 14, S61-S64.
6. **Banki NF, Agota Ver, Laszlo J. Wagner, Adam Vannay, Peter Degrell, Agnes Prokai et al., (2012).** Aldosterone Antagonists in Monotherapy Are Protective against Streptozotocin-Induced Diabetic Nephropathy in rats. PLoS ONE 7(6): e39938. doi:10.1371 /journal. pone.0039938 E
7. **Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, et al. (2004):** Identification of telmisartan as unique angiotensin II receptor antagonist with selective PPAR-modulating activity. Hypertension 43: 993-1002.
8. **Brownlee M. (2001):** Biochemistry and molecular cell biology of diabetic complications. Nature ;414(6865):813-20.
9. **Chang S. K. and Stevens W. C.(1992):** Endothelium-dependent increase in vascular sensitivity to phenylephrine in long-term streptozotocin diabetic rat aorta Br. J. Pharmacol. 107, 983-990M

10. **Chatzigeorgiou A, Halapas A, Kalafatakis K. and Kamper E. (2009):** 'The use of animal models in the study of diabetes mellitus.' In vivo (Athens, Greece); 23(2):245-58.
11. **Cooper SA, Whaley-Connell A, Habibi J, Wei Y, Lastra G, Manrique C, et al. (2007):** Renin-angiotensin- aldosterone system and oxidative stress in cardiovascular insulin resistance.' American journal of physiology. ; 293(4):H2009-23.
12. **De Mattia G, Laurenti O and Fava D, (2003):** 'Diabetic endothelial dysfunction: effect of free radical scavenging in Type 2 diabetic patients. Journal of Diabetes and Its Complications'. 17, 30-35**.**
13. **Desoky N, El-Bassossy HM, Fahmy A. and Azhar A. (2014):** Apigenin restores normal vascular reactivity in diabetic rats via protein kinase C inhibition. Z.U.M.J.Vol.20; N.1;1-5.
14. **Goyal BR, Parmar K, Goyal RK. and Mehta AA. (2011a):** Beneficial role of telmisartan on cardiovascular complications associated with STZ-induced type 2 diabetes in rats. Pharmacological report 63, 956-966.
15. **Goyal BR, Shraddha V. Bhadada, and Mayur M. Patel. (2011b):** Comparative evaluation of spironolactone, atenolol, metoprolol, ramipril, and perindopril on diabetes-induced cardiovascular complications in type 1 diabetes in rats. Institute of Pharmacy, vol. 19 no.1
16. **Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, et al. (1999):** 'Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association.' Circulation; 100(10):1134-46.
17. **Hamed A. A. and Malek H. A. (2007):** Effect of Telmisartan in Experimentally Induced Diabetes Mellitus in Rats. International Journal of Health Sciences, Qassim University, Vol. 1, No.2, 249-256.
18. **Hasan OA. And Al-Hammami FA. (2012):** Comparative Study of Lisinopril Versus Telmisartan Effects on Oxidative Stress in Diabetic Type 2 Hypertensive Patients. Raf. J. Sci., Vol. 23, No.2 pp 34-41.
19. **Heygate K. M, Lawrence I. G, Bennett M. A. and Thurston H.(1995):** Impaired endothelium-dependent relaxation in isolate resistance arteries of spontaneously diabetic rats. Br J Pharmacol. 116(8): 3251–3259
20. **Khan MS. and Gupta AK. (2015):** Hypoglycaemic effect and interactions of ARB (telmisartan) with oral Hypoglycaemic agents in STZ induced diabetic rats. Volume 4, Issue 05, 1505-1515.
21. **Kojima N, Williams JM, Slaughter TN, Kato S, Takahashi T, Miyata N. and Roman RJ. (2015):** Renoprotective effects of combined SGLT2 and ACE inhibitor therapy in diabetic Dahl S rats. Physiol Rep, 3 (7), e12436,1-13.
22. **Maiese K, Morhan SD and Chong ZZ, (2007):** ' Oxidative stress biology and cell injury during type1 and 2 diabetes mellitus.' Current neurovascular research, 4, 63-71.
23. **Mayyas F, Alzoubi KH. and Bonyan R. (2017):** The role of spironolactone on myocardial oxidative stress in rat model of streptozotocin-induced diabetes. Cardiovasc Ther. 35:e12242.
24. **Mohd RM. And Macha A. (2006):** Effects of ascorbic acid on impaired vascular reactivity in aortas isolated from age-matched hypertensive and diabetic rats. Vascular Pharmacology 45 (2006) 127–133.
25. **Mollnau H, Oelze M, Zinßius E, Hausding M, Wu Z, Knorr M, et, al., (2013):** Effects of telmisartan or amlodipine monotherapy versus telmisartan/amlodipine combination therapy on vascular dysfunction and oxidative stress in diabetic rats. Arch Pharmacol 386:405–419
26. **-Moussa SA. (2015):** Oxidative stress in diabetes mellitus. ROMANIAN J. BIOPHYS., Vol. 18, No. 3, P. 225–236.
27. **Pagano P. J., Griswold M.C., Ravel D. and Cohen R.A. (1998):** Vascular action of the hypoglycaemic agent gliclazide in diabetic rabbits Diabetologia 41: 9--15
28. **Paliwal YK, Mehan S, Pijjim VK. and Sharma P. L. (2014):** Renoprotective effect pf lisinopril and hemin combination against streptozotocin induced diabetic nephropathy in diabetic rats. Pharmacologia 60-75.
29. **Polizio HA. and Pena C. (2007):** Lisinopril as an antioxidant in hypertension?. Antioxd. and Red. Sign., 9(3), 394-398.
30. **Ramanadham S, Lyness WS. and Tenner TJ. (1984):** Alterations in aortic and tail artery reactivity to agonists after STZ treatment Canadian Journal of Physiology and Pharmacology 62(4):418-23.
31. **Robertson RP. (2004):** Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes, J. Biol. Chem., 279(41), 42351–42354

32. **Roghani M, Jalali-Nadoushan MR, Baluchnejadmojarad T, Vaez Mahdavi MR, Naderi G, Dehkordi FR. and Joghataei MT. (2013):** Endothelium-dependent Effect of Sesame Seed Feeding on Vascular Reactivity of Streptozotocin-diabetic Rats: Underlying Mechanisms. Iranian Journal of Pharmaceutical Research (2013), 12 (3): 377-385.

33. **Schäfer A, Flierl U, Vogt C, Menninger S, Tas P, Ertl G. and Bauersachs J. (2007):** Telmisartan improves vascular function and reduces platelet activation in rats with streptozotocin-induced diabetes mellitus Pharmacological Research Volume 56, Issue 3, Pages 217–223.

34. **Sena C. M., Louro T. P., Matafome E., Nunes P. and Monteiro R. (2009):** Antioxidant and Vascular Effects of Gliclazide in Type 2 Diabetic Rats Fed High-Fat Diet. Physiol. Res. 58: 203-209.

35. **Senapaty S, Rath B, Jena J. and Biswal SB. (2014):** Comparative Effect Of angiotensin II Type I Receptor Blockers On Blood Glucose Concentration And Oxidative Stress In Streptozotocin-Induced Diabetic Rats.'Int J Pharm Pharm Sci, Vol 6, Issue 6, 266-269.

36. **Silva M. A, NascimentoT. B, Cau S. B, Lopes R. A, Mestriner F. L, Fais R. S, Touyz R. M. and Tostes R C. (2015):** Spironolactone treatment attenuates vascular dysfunction in type 2 diabetic mice by decreasing oxidative stress and restoring NO/GC signaling. Front Physiol. 2015; 6: 269.

37. **Silva M. A, NascimentoT. B, Cau S. B, Lopes R. A, Mestriner F. L, Fais R. S, Touyz R. M. and Tostes R C. (2015):** Spironolactone treatment attenuates vascular dysfunction in type 2 diabetic mice by decreasing oxidative stress and restoring NO/GC signaling. Front Physiol.; 6: 269.

38. **Vallejo S, Angulo J, Piero C, Sanchez A, Cercas E, Liergo J, et. al., (2000):** Prevention of endothelial dysfunction in STZ-induced diabetic rats by gliclazide treatment. Journal of diabetes and its complication Vol, 14, Issue 4, Pages 224–233.

39. **Waanders F, Hoven JM, Rops LA, Kramer BA, Goor H. and Berden HJ. (2008):** Regulation of glumerular heparanase expression by aldosterone, Angiotensin II, and reactive oxygen species. Nephrol. Dial. Transplant., 7, 86-92.

40. **Watanabe T, Barker TA. and Berk BC (2005**): Angiotensin II and the endothelium: diverse signals and effects. Hypertension 2005;45(2):163-9.

41. **Xavier FE, Davel AP, Rossoni LV. and Vassallo DV (2003):** Time-dependent hyperreactivity to phenylephrine in aorta from untreated diabetic rats: role of prostanoids and calcium mobilization. Vascular Pharmacology; 40:67-76.

42. **Yuan J, Jia R. and Bao Y (2007):** Beneficial effects of spironolactone on glomerular injury in streptozotocin-induced diabetic rats. JRAAS 8:118–26.