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BIOCHEMICAL AND PHYSIOLOGICAL STUDY ON THE EFFECT OF LISINOPRIL AND TELMISARTAN ON VASCULAR REACTIVITY AND METABOLIC CHANGES IN DIABETIC RATS

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Abstract

Many edematous illnesses, such as heart disease, liver disease, drug-resistant hypertension, chronic kidney disease, metabolic syndrome, and diabetes mellitus, are associated with a pathogenic role for the renin-angiotensin-aldosterone system (RAAS). The discovery that both aldosterone and angiotensin II (AII) can induce oxidative damage and are profibrotic and proinflammatory has led to the beneficial effects of medications acting on the RAAS, such as aldosterone-receptor antagonists

(ARAs), direct renin inhibitors (DRIs), ACEIs, and angiotensin receptor blockers (ARBs) in renal and cardiovascular diseases (CVD).
The benefits of RAAS blockers in CVD and renal therapy are attributed to multiple processes, including the hemodynamic effects of AII neutralization and the inhibition of AII-dependent tissue production of growth-promoting cytokines, free oxygen radicals, and fibrosis mediators. One of the most effective therapeutic strategies in medicine today is the blockade of the RAAS with ACEIs and ARBs. Patients with hypertension, acute myocardial infarction, chronic systolic heart failure, stroke, and diabetic renal disease have all been demonstrated to benefit from RAAS blockade.The present work aimed to study and evaluate the effects of RAAS inhibitors, ACE inhibitors (lisinopril), and ARB (telmisartan) on the vascular reactivity of the diabetic rats' aortae and the DM-induced biochemical changes.
Results showed that treatment withRAAS blocker ARB (telmisartan), better than ACE inhibitor (lisinopril), significantly improved the acetylcholine-induced vasodilatation and attenuated the noradrenaline-inducedcontraction of the isolated rat aorta in the diabetic rats as compared to diabetic untreated rats. Urea and creatininelevels decreased significantly in the RAAS blockers treated diabetic rats compared with those of the diabeticuntreated rats.
In conclusion, RAAS blockers improvedisturbedrenal functionsandvascularreactivityindiabeticrats.

Keywords: Renin-Angiotensin-Aldosterone System (RAAS)- telmisartan- lisinopril- Diabetes - albino rats

Introduction

Diabetes mellitus (DM) is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion and/or insulin action; despite the availability of different classes of oral hypoglycemic drugs, the incidence of microvascular complications (nephropathy, retinopathy, and neuropathy) and macrovascular complications (atherosclerosis, coronary artery disease, peripheral arterial disease, and stroke) continues to rise unabated in diabetic patients, even with treatment **(Verhulst *et al.*, 2019).**
Evidence implicates the role of oxidative stress in the different stages of the development ofdiabetes mellitus, starting from the pre-diabetes state, impaired glucose tolerance,postprandial hyperglycemia, mild diabetes, and finally overt DM giventhe evidence that implicates a role of oxidative stress in β-cell dysfunction andinsulin resistance, antioxidants could play a role in preventingdiabetes mellitus and/or its progression. Antioxidants such as vitamin C, vitamin E, β-carotene, α-lipoic acids, and honey have been shown to ameliorate hyperglycemia through increased β-cell mass and insulin secretion **(Yang *et al.*, 2011).**
There is strong evidence implicating the role of oxidative stress in diabetic nephropathy, retinopathy, and neuropathy, which constitute microvascular complications **(Kang and Yang *et al.*, 2020).** Similarly, oxidative stress is implicated in macrovascular complications (coronary artery disease, peripheral arterial disease, and cerebrovascular disease).
Understanding the tissue renin-angiotensin-aldosterone system, a crucial factor in the progression of tissue damage in Diabetes mellitus (DM), is imperative for protecting against tissue damage in this chronic disease.

Materials and Methods

Experimental animals:

Adult male albino rats were chosen as an animal model for this study. Rats were brought from the animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, and maintained a balanced diet with a water supply in clean containers. They were kept for two weeks to adapt to the laboratory conditions before the start of the experiment. Sixty age-matchedadult male albino rats with initial

body weights ranging from 150 to 200g were used. The rats were divided into six groups (10 rats each):

Group I: Normal control untreated non-diabetic rats injected intraperitonealwith 0.1 M (pH 4.5) citrate buffer and received 1 ml saline (vehicle) orally.

Group II: Diabetic untreated rats.

Group III: Diabetic rats treated with gliclazide (10mg/kg)dissolved in distilled water once daily orally for eight weeks.

Group IV: Diabetic rats treated with telmisartan (5mg/kg) dissolved in distilled water once daily orally for eight weeks.

Group (V): Diabetic rats treated with lisinopril (10 mg/kg) dissolved in distilled water once daily orally for eight weeks.

Drugs and chemicals:

Acetylcholine (ACH) (Fluka, Switzerland),
Gliclazide (Sigma Aldrich, USA),
Lisinopril (Sigma Aldrich, USA),
Telmisartan (Sigma Aldrich, USA), and
Streptozotocin(STZ) (MP biomedicals, LLC—France).

Procedures:

1- Induction of diabetes

Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight dissolved in 0.1 M (pH 4.5) citrate buffer **(Chatzigeorgiou *et al.*, 2009).** (The control group was given an equivalent volume of citric acid buffer). STZ induces diabetes within two days by destroying β cells. Diabetes was confirmed by detecting blood glucose concentration using the glucose oxidase method using a glucometer with a glucose test strip (One Touch Basic). Two days after STZ injection, rats with more than 250 mg/dl of blood glucose levels were considered diabetic and included in the study **(Chatzigeorgiou *et al.*, 2009).**

2- Collection of blood samples

2.1. The animal was anesthetized with ether by placing the rat in an anesthetic box filled with ether vapor, which was maintained by periodically applying liquid ether to cotton wool on the base of the box. When the surgical stage of anesthesia was reached (judged by loss of withdrawal reflexes), the animal was removed and placed on a table. Blood was collected from the retro-orbital plexus using a capillary tube (0.75-1.0 mm internal diameter) inserted in the medial canthus medial to the eye globe.

2.2. After eight weeks, rats were fasted overnight. They were anesthetized with ether by placing the rat in an anesthetic box filled with ether vapor. It was maintained by periodically applying liquid ether to cotton wool on the base of the box. When the surgical stage of anesthesia was reached (judged by loss of withdrawal reflexes), the animal was removed and placed on a table. Blood was collected from the carotid artery into a dry-clean graduated glass centrifuge tube. The blood samples were allowed to clot at room temperature before centrifugation at 5000 r.p.m for 10 minutes.The collected sera were frozen at –20 $^{\circ C}$until the time of the assay

3- Preparation of the isolated aortic rings

After blood samples were collected, animals were killed by decapitation, and abdominal and thoracic walls were opened. The thoracic aorta was dissected and cut, placed in dish containing Kreb's-Henseleit solution of the following composition (mM/L) :(NaCl 118.4, KCl 4.69, kH2PO4:1.17, MgSO4 1.18, CaCl 2.52, glucose 11.10 and NaHCO3 25) aerated with carbogen (95% oxygen and 5% carbon dioxide), cleaned from the surrounding attached tissues and cut into small rings (about 4mm length).

The aortic rings were suspended in an isolated organ bath (30 ml capacity) containing Kreb's-Henseleit solution maintained at 37°C and aerated with carbogen. Aortic rings were subjected to an initial tension of 1g and were kept in the organ bath (for equilibration) for approximately 90 minutes; the physiological solution was renewed every 15 minutes. Response of the aortic rings to drugs was measured isometrically with a Grass FT O3 force-displacement transducer and recorded on a polygraph. The viability and stability of the tissue were checked by two equal contractile responses to the same concentration of norepinephrine (10^{-7}). Norepinephrine is diluted in 1% Hcl solution to prevent auto-oxidation. Tissues were then washed several times and allowed to relax to baseline level.

Different concentrations of norepinephrine (1×10^{-8} to 1×10^{-3} and 3×10^{-8} to 3×10^{-3}) were prepared and used. Cumulative dose-response curves to norepinephrine were performed on each ring. During the dose-response curves of norepinephrine, each dose was added after reaching the plateau of the response of the previous dose. Each ring was serially washed and equilibrated after obtaining the maximum response to baseline.

For the relaxation study, aortic rings were pre-contracted by a submaximal dose of norepinephrine (10^{-6}). When the response reached its plateau, cumulative concentration-response curves of acetylcholine (1×10^{-8} to 1×10^{-3} and 3×10^{-8} to 3×10^{-3}) were done. Each ring was serially washed after obtaining the maximum response to reach the baseline and equilibrated. During the dose-response curves of acetylcholine, each dose was added after reaching the plateau of the response of the previous dose.

4-Biochemical measurements:

A- Blood glucose measurements:

The enzymatic colorimetric method determined the blood glucose level (**Trinder & Ann, 1969).**Using diamond diagnostic kits

B- Serum insulin level

Serum insulin was determined by an enzyme-linked immunosorbent assay (ELISA) kit **(Csont, 2007).**

C -Lipid Profile

1 - Serum cholesterol measurements

Serum cholesterollevel was measured using the enzymatic-colorimetric method (**Ellefson &Caraway, 1976**). Egyptian company for biotechnology-Egypt.

2 - Serum triglyceride measurements

Serum triglycerides were estimated using an enzymatic colorimetricmethod (**Bucolo& David, 1973**). Egyptian company for biotechnology-Egypt.

3 - Determination of Serum High-Density Lipoproteins

The precipitation method estimated serum high-density lipoprotein (HDL) **(Friedewaled, 1972).** Egyptian company for biotechnology-Egypt.

4 -Determination of Serum Low-Density Lipoproteins

The serum LDL-cholesterol was estimated according to (the **Fried Ewald formula 1972)** using the following equation: -

LDL in (mg/dl) = **Total cholesterol – Triglyceride/5-HDL**

D- Renal function tests

1 - Serum urea measurements

The serum urea level was measured using the colorimetric method **(Batton & Crouch, 1977).** An Egyptian company provided the biotechnology kits.

2 - Serum creatinine measurements

Serum creatininelevel was measured by kinetic method **(Young, 1995).** Biolabo reagents kits – France.

5- Statistical analysis:

Statistical analysis was done using the computer program (SPSS). The quantitative data were presented as mean ± standard error (SE). Data analysis was performed using a one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test to determine the differences between the means. A value of $P < 0.05$ was used as a criterion for statistical significance.

Results

Effect of gliclazide, telmisartan, and lisinopril on the contractile responses of the diabetic rat's aortae to norepinephrine.

The results showed that the contractile response of the aortae to NE increased significantly ($P< 0.001$) in the diabetic untreated rats in comparison with the normal rats.

In the diabetic rats treated with gliclazide, the contractile response of the aortae to NE decreased significantly **($P< 0.01$)** in comparison with the diabetic untreated rats. However, the contractile response values of the glyclazide group didn't return to normal and showed a significant increase **($P< 0.05$)** in comparison to the normal control rats' contractile responses.

Similar to gliclazidetreated group both telmisartan and lisinopril treated groups showed significant decrease in the contractile response of aortae to NE versus diabetic untreated rats**($P< 0.01$),** alsotheir values didn't return to normal and revealed significant difference when compared to normal control group **($P< 0.01$).** Telmisartan is more effective in reducing the contractile responses than both gliclazide andlisinopril but this is not significant statistically {table (1) & fig (1)}

Table (1): Effects of pretreatment with gliclazide, telmisartan, and lisinopril on the contractile response of the diabetic rat's aortae to norepinephrine

Groups	**Normal control**	**Diabetic untreated**	**Diabetic treated with gliclazide**	**Diabetic treated with telmisartan**	**Diabetic treated with lisinopril**
-Log. molar conc. of NE	***Contraction(g)***	***Contraction(g)***	***Contraction(g)***	***Contraction(g)***	***Contraction(g)***
$1x10^{-8}$	**0.00 ± 0.00**	**0.6 ± 0.05***	**0.23±0.03 *#**	**0.14±0.03*#**	**0.28 ± 0.03 *#**
$3x10^{-8}$	**0.06 ± 0.02**	**0.93 ± 0.03***	**0.32 ± 0.04 *#**	**0.26 ± 0.03*#**	**0.43 ±0.03 *#**
$1x10^{-7}$	**0.29 ±0.03**	**1.2±0.04***	**0.68±0.04 *#**	**0.62±0.04 *#**	**0.81 ± 0.05 *#**
$3x10^{-7}$	**0.61 ± 0.05**	**1.44 ± 0.06***	**0.99 ± 0.04 *#**	**0.92 ± 0.04 *#**	**1.08 ± 0.04 *#**
$1x10^{-6}$	**0.89±0.06**	**1.79 ± 0.09***	**1.29 ±0.06 *#**	**1.25 ±0.05 *#**	**1.35 ± 0.06 *#**
$3x10^{-6}$	**1.2 ± 0.06**	**2.11±0.06***	**1.61 ± 0.05 *#**	**1.5 ± 0.04 *#**	**1.65 ± 0.04 *#**
$1x10^{-5}$	**1.6±0.07**	**2.51±0.08***	**2.04 ± 0.07 *#**	**2.05 ± 0.07*#**	**2.07 ± 0.08 *#**
$3x10^{-5}$	**1.6±0.07**	**2.51±0.09***	**2.04±0.08 *#**	**2.05±0.08 *#**	**2.07 ± 0.07 *#**

* Significant difference from the normal control untreated rats ($P< 0.05$)

#Significant difference from the diabetic untreated rats ($P< 0.05$)

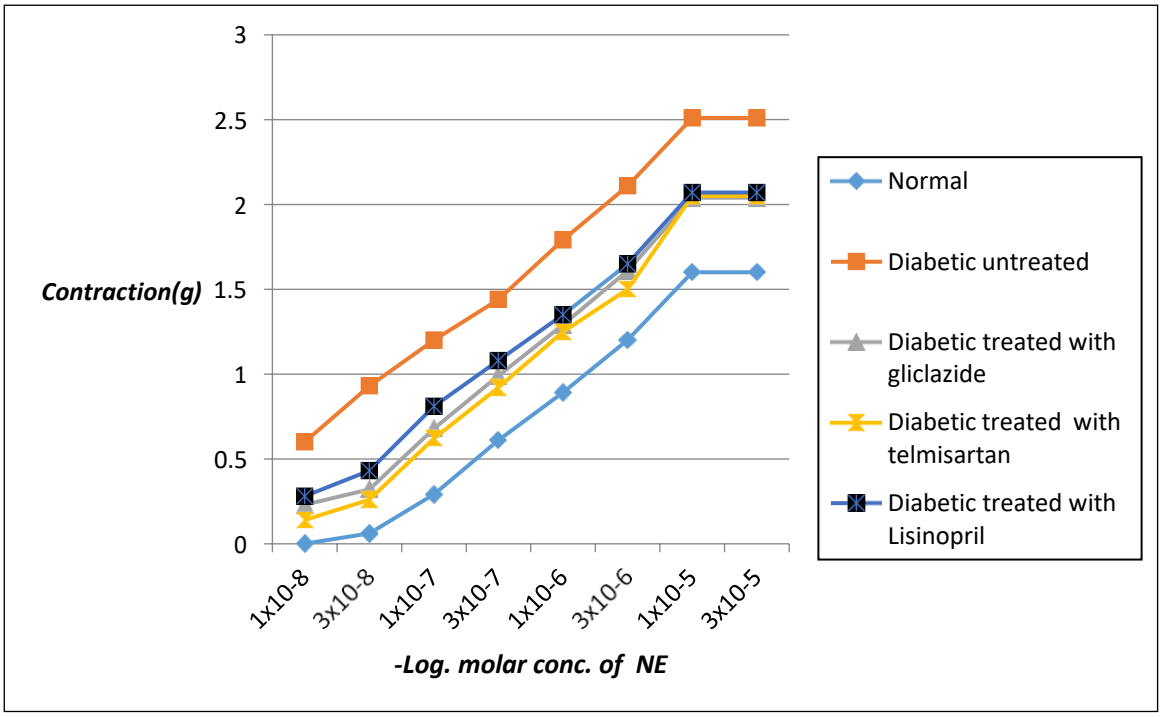

Figure (1): Effect of pretreatment with gliclazide, telmisartan, and lisinopril on the contractile responses of the diabetic rat's aortae to norepinephrine for eight weeks

Effect of gliclazide, telmisartan, and lisinopril on the on the relaxant response of the diabetic rat's aortae to acetylcholine:

The results showed that the relaxant response of the aortae to acetylcholine was decreased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal control rats.

In the diabetic rats treated withgliclazidethe relaxant response to acetylcholine increased significantly (P< 0.01) in comparison with the diabetic untreated rats. However, this relaxant response to acetylcholine in glyclazide group didn't return to normal values and showed a significant decrease (p< 0.05) in comparison to the normal control rats' relaxant responses.

Similar to gliclazidetreated group both telmisartan and lisinopril treated groups showed significant increase in the relaxant response of the aortae to acetylcholine versus diabetic untreated rats **(P< 0.01),** alsotheir values didn't return to normal and revealed significant difference when compared to normal control group **(P< 0.01).** Telmisartan is more effective in increasing the relaxant responses than glyclazide and lisinopril, but this increase is insignificant statistically {table (2)}.

Table (2): Comparison between effects of pretreatment with gliclazide, telmisartan, and lisinopril, with normal and Diabetic untreated groups on the relaxant response of the diabetic rat's aortae to acetylcholine for eight weeks

Groups	Normal	Diabetic untreated	Diabetic treated with gliclazide	Diabetic treated with telmisartan	Diabetic treated with Lisinopril
-Log. molar conc. of Ach	*Relaxation (% of residual tone)*	*Relaxation (% of residual tone)*	*Relaxation (% of residual tone)*	*Relaxation (% of residual tone)*	*Relaxation (% of residual tone)*
$1x10^{-8}$	15.6 ± 1.4	0.0 ± 0.0 *	7.3 ± 1.4 * #	8.7 ± 1.3 * #	7.5 ± 1.3 * #
$3x10^{-8}$	27.4 ± 1.3	2.8 ± 1.1 *	13.5 ± 1.4 * #	19.4 ± 1.3 * #	16.6 ± 1.2 * #
$1x10^{-7}$	44.3 ± 1.2	14.9 ± 1.2 *	26.7 ± 1.3 * #	34.2 ± 1.5 * #	29.4 ± 1.5 * #
$3x10^{-7}$	57.3 ± 1.2	24.7 ± 1.3 *	40.3 ± 1.4 * #	46.5 ± 1.6 * #	43.6 ± 1.4 * #

$1x10^{-6}$	**69.5 ± 1.4**	**31.6 ± 1.3 ***	**49.6 ± 1.4 * #**	**57.9 ± 1.5 * #**	**53.2 ± 1.5 * #**
$3x10^{-6}$	**73.2 ± 1.5**	**37.4 ± 1.4 ***	**53.8 ± 1.5 * #**	**62.8 ± 1.7 * #**	**56.3 ± 1.6 * #**
$1x10^{-5}$	**76.6 ± 1.3**	**43.1 ± 1.4 ***	**57.2 ± 1.6 * #**	**65.4 ± 1.4 * #**	**59.6 ± 1.5 * #**
$3x10^{-5}$	**76.6 ± 1.2**	**43.1 ± 1.2 ***	**57.2 ± 1.4 * #**	**65.4 ± 1.5 * #**	**59.6 ± 1.3 * #**

* Significant difference from the normal control untreated rats (P< 0.05)
#Significant difference from the diabetic untreated rats (P< 0.05)

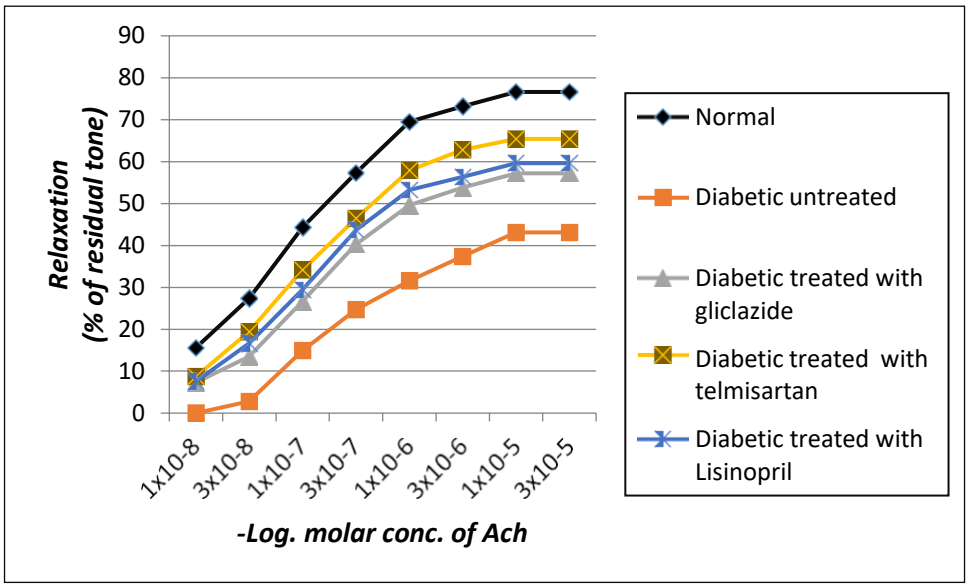

Fig (2): Comparison between effects of pretreatment with gliclazide, telmisartan, and lisinopril, with normal and Diabetic untreated groups on the relaxant response of the diabetic rat's aortae to acetylcholine for eight weeks

Comparison between Effects of treatment with gliclazide, telmisartan, and lisinopril on fasting blood glucoselevel of diabetic rats for eight weeks

The results showed that fasting blood glucose was increased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal rats.

The lowest blood glucose level amongst treated groups was in gliclazide group. In the diabetic rats treated with eithergliclazide or telmisartan, fasting blood glucose level decreased significantly (P< 0.01) compared to untreated diabetic rats. However, the blood glucose level of both groups didn't return back to normal and there was still a significant (P< 0.05) increase compared to the normal rats. On the other side, the blood glucose level of diabetic rats treated with lisinopril showed significant increase in comparison to normal control rats (P< 0.01) and insignificant difference versus diabetic untreated rats (P> 0.05), as shown in Table (3)**.**

Comparison between Effects of treatment with gliclazide, telmisartan, and lisinopril on serum insulin level of diabetic rats for eight weeks

Serum insulin level was decreased significantly (P< 0.001) in the diabetic untreatedrats compared to the normal rats.

The highest serum insulin level amongst treated groups was in gliclazide group.In the diabetic rats treated with with either gliclazide or telmisartan, serum insulin level was increased significantly **(P< 0.01)** compared to the diabetic untreated rats. However, the serum insulin levels of both groups didn't return to normal valuesand showed a significant decrease**(P< 0.05)** compared to the insulin level of normal control rats. On the other side, the serum insulin level of diabetic rats treated with lisinopril showed significant decrease in comparison to normal control rats (P< 0.01) and insignificant difference versus diabetic untreated rats (P> 0.05), as shown in Table (3).

Comparison between effects of treatment with gliclazide, telmisartan, and lisinopril on serum total cholesterollevel of the diabetic rats

The results observed that serum cholesterol was increased significantly **(P< 0.01)** in the diabetic untreatedrats in comparison with the normal rats.

The lowest serum cholesterol level amongst treated groups was in gliclazide group. In this group, serum cholesterol decreased significantly **(P< 0.01)** compared to the diabetic untreated rats. However, there was still a significant increase **(P< 0.05)** compared to the normal control rats.

Also the other treated groups, either telmisartan or lisinopril group showed significant decrease in serum cholesterol levels compared to the diabetic untreatedrats but the cholesterol level didn't return to normal values in both groups and there was still a significant increase **(P< 0.01)** compared to the cholesterol levels in normal control rats. However, the recorded serum cholesterol level of telmisartan group is lower than its value in lisinopril treated group, as shown in Table (3).

Comparison between effects of treatment with gliclazide, telmisartan, and lisinopril on serum triglycerideslevel of the diabetic rats for eight weeks

The results recorded that serum triglycerides were increased significantly **(P< 0.01)** in the diabetic untreatedrats compared to the normal rats.

The lowest serum triglycerides level amongst treated groups was in gliclazide group. In this group, serum triglycerides decreased significantly **(P< 0.01)** compared to the diabetic untreated rats. However, there is still a significant **(P< 0.05)** increase compared to the normal rats.

Also the other treated groups, either telmisartan or lisinopril group showed significant decrease in serum triglycerides levels compared to the diabetic untreated rats but the triglycerides level didn't return to normal values in both groups and there was still a significant increase **(P< 0.01)** compared to the triglycerides levels in normal control rats. However, the recorded serum triglycerides level of telmisartan group is lower than its value in lisinopril treated group, as shown in Table (3).

Comparison between Effects of treatment with gliclazide, telmisartan, and lisinopril on serum HDLlevel of diabetic rats for eight weeks

The results recordedthat serum HDL was decreased significantly **(P< 0.01)** in the diabetic untreatedrats in comparison with the normal rats.

The highest serum HDL level amongst treated groups was in gliclazide group. In this group, serum HDL was increased significantly **(P< 0.01)** compared to the diabetic untreated rats. However, there was a significant **(P< 0.05)** decrease as compared to the normal rats, as shown in Table (3).

Also the other treated groups, either telmisartan or lisinopril group showed significant increase in serum HDL levels compared to the diabetic untreated rats but the HDL level didn't return to normal values in both groups and there was still a significant decrease **(P< 0.01)** compared to the HDL levels in normal control rats. However, the recorded serum HDL level of telmisartan group is higher than its value in lisinopril treated group, as shown in Table (3).

Comparison between effects of treatment with gliclazide, telmisartan, and lisinopril on serum LDLlevel of the diabetic rats for eight weeks

The results recorded that serum LDL was increased significantly **(P< 0.01)**in the diabetic untreatedrats in comparison with the normal rats.

The lowest serum LDL level amongst treated groups was in gliclazide group.In this group, serum LDL decreased significantly **(P< 0.01)** compared to the diabetic untreated rats. However, there was a significant increase**(P< 0.05)** as compared to the normal rats, as shown in Table (3).

Also the other treated groups, either telmisartan or lisinopril group showed significant decrease in serum LDL levels compared to the diabetic untreated rats but the LDL level didn't return to normal in both groups and there was still a significant increase in their levels **(P< 0.01)** compared to the LDL level in normal control rats. However, the recorded serum LDL level of telmisartan group is lower than its value in lisinopril treated group, as shown in Table (3).

Comparison of treatment effects with gliclazide, telmisartan, and lisinopril on the serum urea level of the diabetic rats for eight weeks.

The results recorded that serum urea was increased significantly **(P< 0.01)** in the diabetic untreatedrats compared to the normal control rats.

The lowest serum urea level amongst treated groups was in gliclazide group.In this group, serum urea was decreased significantly **(P< 0.01)** in comparison with the diabetic untreated rats; However, there was a significant increase**(P< 0.05)** as compared to the normal rats serum urea.

Also the other treated groups, either telmisartan or lisinopril group showed significant decrease in serum urea levels compared to the diabetic untreated rats but the urea level didn't return to normal in both groups and there was a significant increase in their levels **(P< 0.01)** compared to the urea level in normal control rats. However, the recorded serum urea level of telmisartan group is lower than its value in lisinopril treated group, as shown in Table (3).

Comparison between effects of treatment with gliclazide, telmisartan, and lisinopril onserum creatinine level of the diabetic rats for eight weeks

The results show that serum creatininewas increased significantly **(P< 0.01)** in the diabetic untreatedrats compared to the normal control rats.

The lowest serum creatinine level amongst treated groups was in gliclazide group.In the diabetic rats treated with gliclazide, serum creatininedecreased significantly **(P< 0.01)** compared to the diabetic untreated rats. However, there was a significant **(P< 0.05)** increase as compared to the normal rats, as shown in Table (3).

Also the other treated groups, either telmisartan or lisinopril group showed significant decrease in serum creatinine levels compared to the diabetic untreated rats but the creatinine level didn't return to normal in both groups and there was a significant increase in their levels **(P< 0.01)** compared to the creatinine level in normal control rats. However, the recorded serum creatininelevel of telmisartan group is lower than its value in lisinopril treated group, as shown in Table (3).

Table (3): Levels of measured biochemical parameters in normal control, Diabetic untreated, Diabetic treated with gliclazide, telmisartan and lisinopril

	Normal control	Diabetic untreated	Diabetic treated with gliclazide	Diabetic treated with telmisartan	Diabetic treated with lisinopril
Fasting blood glucose level(mg/dl)	**86.32 ± 3.8**	**289.12 ± 5.82 ***	**129.04 ± 3.57 *#**	**178.7 ± 4.08*#**	**279.21 ± 7.26 ***
Serum insulin level (µIU/ml)	**6.13 ± 0.24**	**2.56 ± 0.13 ***	**4.96 ± 0.17 *#**	**3.78 ± 0.19 *#**	**2.67 ± 0.18 ***
Serum cholesterol (mg/dl)	**95.86 ± 199**	**203.51 ± 3.19 ***	**119.2 ± 1.72 *#**	**134.99 ± 1.82 *#**	**163.12 ± 1.78 *#**
Serum triglycerides (mg/dl)	**67.86 ± 1.54**	**172.8 ± 2.77 ***	**91.4 ± 1.76 *#**	**107.14 ± 1.78 *#**	**124.32 ± 1.58 *#**
Serum HDL (mg/dl)	**49.5 ± 1.88**	**17.3 ± 1.12 ***	**37.88 ± 1.29 *#**	**35.04 ± 1.23 *#**	**32.65 ± 1.85*#**
Serum LDL (mg/dl)	**74.09 ± 3.72**	**183.65 ± 5.29 ***	**100.02 ± 3.42 *#**	**113.51 ± 3.32*#**	**129.81 ± 2.46 *#**
Blood urea (mg/dl)	**23.35 ± 1.63**	**92.55 ± 2.73 ***	**46.2 ± 1.21 *#**	**52.54 ± 2.23*#**	**60.22 ± 1.74 *#**
Serum creatinine (mg/dl)	**0.79 ± 0.05**	**2.23 ± 0.1 ***	**1.25 ± 0.05 *#**	**1.31 ± 0.07*#**	**1.5 ± 0.09*#**

* Significant difference from the normal control untreated rats (P< 0.05)

[#]Significant difference from the diabetic untreated rats ($P< 0.05$)

Discussion

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both**.** DM affects approximately 170 million individuals worldwide and is expected to alter the lives of at least 366 million individuals within a future span of 25 years **(Kharroubi & Darwish, 2015)**

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 **(Verhulst *et al.*, 2019).**

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 **(Teodoro*et al.*, 2019).**

The renin-angiotensin system plays a crucial role in circulatory homeostasis and the regulation of vascular tone. There is a growing body of evidence that enhanced activation of RAAS and the subsequent increase of AII and aldosterone levels contribute to changes in 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 the development of diabetic complications**(Li *et al.*, 2012).**

The present study showed that the contractile response of the rat's isolated aortae induced by norepinephrine (NE) was increased significantly in the diabetic untreated rats compared to the normal rats. 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; these results are in agreement with **Desoky *et al.*, 2014** which reported that diabetes was associated with deterioration in vascular reactivity with significant increases in aorta responsiveness to phenylephrine (PE) and to KCl and a large decrease in aorta responsiveness to ACh. **Xavier *et al.*, 2003** reported that STZ-induced diabetes produced enhanced responsiveness to PE in aortae, although evidencing an increased production of endothelium-derived NO.

Noradrenaline, a neurohormone and sympathetic neurotransmitter, is responsible for major changes in vascular tone and regional blood flow**. (Tran et al., 2022)** Acting via adrenoceptor noradrenaline elicits a range of actions, including direct vasoconstriction, direct vasodilatation, or attenuation of vasoconstrictive and vasodilator agents. Noradrenaline, released from the postganglionic fibers at the nerve terminals and circulating catecholamines, acts through α- and β-adrenoceptors (ARs) **(Carbajal-García et al., 2020).** In the periphery, α- and β-adrenoceptors cause mainly constriction and dilatation, respectively. The actions of noradrenaline on the resistance vasculature are mainly mediated by α1- and α2-adrenoceptors on smooth muscle cells. Noradrenaline can be given to a particular organ to study the direct effect(s) in smooth vascular muscles of a particular blood vessel. **(Archer et al., 2021)**

Apoptosis or programmed cell death can be induced by in vitro exposure of cultured vascular smooth muscle cells to the conditioned medium**(Aravani et al., 2020)**. Hormones and/or substrate contributing to the formation of the final component of neurohormonal response serve as "guppy in each bowl" like COX metabolites present in plasma usually contribute to the formation of vasomotor tone and reactivity in the systemic vasculature as well as in its different vascular trees during diseases/hypodynamia, age, and diabetes **(Imig, 2020)** A complex interaction of these distinct components elicits a full neuro-humoral response. Many factors such as age, gender, function of endocrine glands, and systemic diseases, including diabetes, also influence the vascular reactivity to noradrenaline, among other agonists. The role of insulin deficiency and its treatment in modifying the impact of diabetes on vasculature is quite pertinent **(Li et al., 2023).**

The results of the present study showed that the contractile response of the aorta induced by NE is decreased significantly in the diabetic rats treated with gliclazide compared 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 gliclazide compared with the diabetic untreated rats.

Similar to gliclazidetreated group Both temisartan and lisinopril groups showed significant decrease in contractile response to NE versus diabetic untreated rats but both groups didn't return back to normal.**Also,**telmisartan and lisinopril treated groups showed significant increase in the relaxant response of the aortae to acetylcholine versus diabetic untreated but their values didn't return to normal .

The results obtained from the present study agree with those obtained by other workers; **Tentolouris *etal.*, 2020** reported that gliclazide treatment improved endothelium-dependent vascular relaxation in diabetic rats. These beneficial effects on the vasculature may be related to the drug's metabolic actions, to improvements in plasma lipids and fasting glycemia, and to its antioxidant properties.

The present work showed that fasting blood glucose increased significantly and serum insulin level decreased significantly in diabetic untreated rats compared to normal rats. In the diabetic rats treated with gliclazide, fasting blood glucose decreased significantly. Serum insulin levels increased significantly in comparison with the diabetic untreated rats. This finding agreed with the results obtained by **MOHARIR *et al.*, 2020**who showed that STZ induced a significant elevation in serum glucose concomitant with a significant reduction of serum insulin compared to the control counterpart. Treatment with gliclazide resulted in a significant reduction of serum glucose with a significant rise in serum insulin.

Similar to gliclazide,the present study reported that telmisartan treated rats showed significant decrease in fasting blood glucose level and significant increase in serum insulin level in comparison with the diabetic untreated rats.On the other side, lisinopril has no significant effect on blood glucose or serum insulin levels. These results agree with **Ajani and Ibrahim's 2020**, they reported that treatment with lisinopril did not affect the elevated serum glucose concentration in diabetic rats. On the other hand, the results of the present study were in disagreement with the results obtained by **Agrawal and Gupta 2013. They** mentioned thatlisinopril has shown significant anti-hyperglycemic effects in diabetic rats and it enhanced the hypoglycemic activity of oral anti-diabetic drugs (Metformin, Gliclazide, and Pioglitazone) in a highly significant manner. They suggested that lisinopril may have some insulin sensitivity potentiating properties in T2DM but not in normoglycemic rats. Though the mechanism for this drug-induced hypoglycemia is not well defined, it is proposed that the increase in bradykinins associated with ACE inhibitor use may cause an increase in insulin sensitivity **(Agrawal & Gupta, 2013**).The differences observed in these results may be due to the difference in the dose and duration of the drug treatment schedule or the severity of hyperglycemia.

The present study showed an increase in serum concentrations of total cholesterol, triglycerides, and LDL, and a consequent decrease in HDL levels was noted in diabetic rats compared to normal rats. Treatment with gliclazide significantly attenuated diabetes-induced alteration in lipid levels, and these results agreed with**Burgeiro*et al.*, 2017** reported that diabetic rats showed a significant increase in fasting glycemia, total and non-HDL-cholesterol, triglycerides, and FFA levels when compared with normal rats receiving a standard diet. Treatment of the animals with 10 mg/kg gliclazide in drinking water significantly decreased fasting glycemia, total and non-HDL cholesterol, and FFA levels.

In addition the present study recorded that total cholesterol, serum triglycerides, and LDL decreased significantly, while HDL was increased significantly in the diabetic rats treated with either telmisartan or lisinopril.Telmisartan showed lower values of total cholesterol, serum triglycerides, and LDL and higher value of HDL than lisinopril. These results are in agreement with those obtained by **Seedevi *et al.*, 2020**, who reported that diabetic rats showed increased serum TG, LDL, and/or total cholesterol levels. The treatment with telmisartan caused a significant improvement in all these parameters.

There are several benefits of RAAS blockade in this condition, such as preserving cellular potassium and magnesium, which leads to better insulin action. This is related to the reduction of sympathetic overactivity or even because of the direct effect of RAAS blockade on insulin signaling, glucose transporters, and the promotion of adipocyte differentiation. Telmisartan is a partial agonist of PPARγ and affects the expression genes responsible for carbohydrate and lipid metabolism, reducing glucose, insulin resistance, and lipid levels in obese rats **(Ohbayashi *et al.*, 2010).**In this context, Toyoma et al. (2011) suugested that the role of telmisartan in improving the vascular reactivity of diabetic rats is mediated by its PPARγ activation and the associated anti-inflammatory effect of telmisartanby attenuation of vascular nuclear factor kappa B (NFκB) activation and tumor necrosis factor α.

The present study results are in line with the results of the clinical study by **Derosa *et al.*, 2004** who reported that telmisartan treatment at a dose of 40 mg once daily for 12 months resulted in a significant reduction in plasma LDL, total cholesterol, and TG levels as compared with baseline. While in the patients treated with eprosartan (the first non-biphenyl, non-tetrazole ARB to be used clinically) 600 mg once-daily, lipid levels were similar to those observed in placebo-treated patients maintained on a strict diet with restricted fat intake.

The present study showed that blood urea and serum creatinine were significantly increased in the diabetic untreatedrats compared with the normal rats. In contrast, in diabetic rats treated with gliclazide, serum urea, and creatinine decreased significantly compared with those in untreated diabetic rats.

These results are in agreement with those obtained by **El-Hazmi *et al.* 2020**. They reported that serum urea, creatinine, and albuminuria were significantly higher in the diabetic untreated group than those in the control group. Gliclazide treatment resulted in significant reductions in the aforementioned parameters.

The RAAS is an important pathway of progression in cardiovascular disease, diabetic nephropathy, and chronic renal disease through inflammation, fibrosis, and necrosis. For this reason, ACE inhibitors and ARBs are effective in the treatment of chronic heart failure and diabetic nephropathy **(Bernardi*et al.*, 2016).**

The results of the present study showed that treatment of diabetic rats with either telmisartan or lisinopril significantly decreased blood urea and serum creatinine levels as compared to diabetic untreated rats. These results agree with the results of **Khan and Imig (2011), who** reported that Telmisartan demonstrated potent nephroprotective effects in different rat models of diabetes and hypertension. In addition, the progressive increase in urinary protein and albumin excretion observed in untreated diabetic rats was dose-dependently inhibited by long-term treatment with telmisartan or lisinopril. With the higher dose of telmisartan and lisinopril, these parameters were almost reduced to values observed in the normoglycemic rats.

Conclusion

The previous results showed that both RAAS blocker (Telmisartan) andACE inhibitor (lisinopril) have significant effects in improving disturbed renal functions,vascularreactivity and other biochemical markers related to the pathophysiology of diabetes in diabetic rats and also showed that Telmisartan is slightly more beneficial than lisinopril regarding these effects.

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