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RESEARCH ARTICLE
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STUDY OF THE EFFECT OF TREATMENT WITH THE RENIN-ANGIOTENSIN SYSTEM INHIBITOR LOSARTAN AND THE LIPID-LOWERING DRUG ATORVASTATIN AND THEIR INTERACTIONS IN DIABETIC RATS

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Abstract

Background: Diabetes mellitus (DM) is a chronic metabolic disorder distinguished by high levels of blood sugar and disruptions in lipid, carbohydrate & protein metabolism due to insulin insufficiencies.

Aim: This study evaluated the effectiveness of Renin-Angiotensin-Aldosterone System antagonist losartan&its combination with atorvastatin in preventing diabetes mellitus accumulations.

Methods: The animal utilized in this study consisted of80 adult male albino ratsweighed between 200 and 250gfed a well-balanced diet and provided with clean water in containers. Before starting the

experiment, they were housed for one week to acclimate to the laboratory environment.In this study, rats were divided into 8 groups and diabetes was induced by a single dosage of fifty mg/kgof an intraperitoneal injection of streptozotocin.
Isolated aortic rings were performed. Blood samples were collected for measuring of tumor necrosis factor-alpha level,lipid profile,serum insulin, blood glucose level, andrenal function tests:Serum creatinine measurements &Serum uric acid measurementsat the end of experimental period.

Results: The contractile response of the aorta induced by norepinephrine is significantly diminished in diabetic rats receiving glimepiride or losartan compared to diabetic rats untreated.The same treatment caused a significant ($P<0.001$) decrease in tumor necrosis factor-alpha (TNF-α) level, blood glucose level in addition to a significant increase in serum insulin level.Furthermore, there has been an improvement in oxidative stress parameters and renal function.**Conclusion:** RAAS blocker losartan and atorvastatin ameliorated SZT-induced diabetic nephropathy, impairment of lipid profile, and endothelial dysfunction through antihypertensive, antioxidant, and anti-inflammatory activities, and can protect against diabetic-induced renal and cardiovascular complications.

Keywords: renin-angiotensin; lipid-lowering drug; diabetic; rats.

Introduction

DM is a metabolic disorder that is consideredasirregularities in the metabolism of lipids, carbohydrates, & proteins. Additionally, diabetes mellitus is typically characterized by continuously high blood sugar levels. Diabetes mellitus is a condition that is caused by multiple factors. Insufficiencies in the secretion and/or activity of insulin are the primary cause of these disorders.**(1)**

Given the evidence relating oxidative stress to insulin resistance & β-cell dysfunction, antioxidants may have the potential to prevent the development and/or progression of diabetes mellitus. Previous studies have demonstrated that antioxidants, involving honey, vitamin E, vitamin C, β-carotene, & α-lipoic acids, can alleviate hyperglycemia by increasing most β-cells & secreting insulin.**(2)**

It has been shown that atorvastatin inhibits the production of superoxide in isolated mice vascular smooth muscle cells by impeding the activation of NADPH oxidase induced by Ang II. Additionally, atorvastatin stimulates the expression of heme oxygenase 1, an enzyme known for its anti-inflammatory, antioxidant, & anti-apoptotic properties. Conversely, in cases with hypercholesterolemia , treatment with atorvastatin induces insulin resistance in a dose-dependent way & elevates their risk of developing type-2 diabetes**.(3)**

To prevent cardiovascular disorder, it may be crucial to devise optimal management strategies for cases with hypercholesterolemia, hypertension, metabolic syndrome, diabetes, or obesity using combined therapy involving statins and RAS inhibitors**.(4)**
The objective of this research was to assess the efficacy of RAAS antagonist losartan,as well as its combination with the hypolipidemic atorvastatin, in preventing accumulations induced by diabetes mellitus.

Materials and Methods

The animal model utilized in this investigation consisted of80 adult male albino ratsweighed between 200 and 250g. Rats originating from the animal house at the Faculty of Medicine, Assiut University, Assiut, Egypt, were provided with a hygienic environment and unrestricted access to clean water containers while consuming a balanced diet. Before commencing the experiment, they were housed for one week to adjust to the laboratory environment.
Using the approach outlined by **Paget and Barnes (1964) (5),** which computed the dose in relation to the animal's surface area, the doses of the tested medications were determined. These doses were

selected as the rodent equivalent to the human therapeutic dose.. Rats were weighed at the end of every week and doses (mg/kg) of the drugs were calculated according to the last recorded body weight. Drug solutions were given orally (P.O) once every day using an oral gavage tube.

Rats were assigned into the following eight groups (ten rats in each):
Group I: rats without diabetes were administered a solution of 0.1 M citrate buffer (pH 4.5) and 1 ml saline (vehicle) orallyfor eight weeks.
Group II:rats with diabetes received distilled waterfor eight weeks.
Group III:rats with diabetes were administered glimepiride orally once daily at a dosage of 0.5 mg/kgfor eight weeks**.**
Group IV:rats with diabetes were administered losartan at a dosage of 2 mg/kg, dissolved in distilled water, once daily orally for eight weeks**.**
Group Vrats with diabetes were administered atorvastatin at a dosage of 10mg/kg, dissolved in distilled water, once daily orally for eight weeks.
Group VI:rats with diabetes were administered glimepiride (0.5mg/kg) and losartan (2mg/kg) orally once daily for eight weeks**.**
Group VII:rats with diabetes were administered glimepiride (0.5mg/kg) &atorvastatin (10mg/kg) orally once daily for eight weeks.
Group VIII: rats with diabetes were administered a daily oral dose oflosartan (2 mg/kg) and atorvastatin (10mg/kg) for eight weeks.

Procedures:
Induction of diabetes: A single intraperitoneal injection of streptozotocin at a dosage of fifty mg/kgof body weight was administered to each animal **(6)**. Overnight, the animals were provided with a five percent glucose solution to alleviate the hypoglycemia induced by the drug **(7).** Diabetes was confirmed through the utilization of a glucometer equipped with a glucose test strip (One Touch Basic) to measure blood glucose concentration utilizing the glucose oxidase method **(8).** Diabetic status was established in the animals when their blood glucose levels surpassed 250 milligrams per deciliter on the third day following streptozotocin injection **(9).** On the 4th day following streptozotocin injection, treatment commenced; this date was designated as the first day oftherapy. Continuation of therapy lasted for eight weeks **(10).**
Collection of blood samples: A-The rats were given ether anesthesia by placing them in an anesthetic box that contained ether vapor and then securely locking the box. At regular intervals, liquid ether was injected into a cotton wool pad that was placed at the bottom of the box. This allowed the anesthesia to be maintained consistently. After eight weeks, rats were allowed to fast for an entire night, & blood samples were taken from the carotid artery followingthe animals were sacrificed. After being collected, the blood was placed in a graduated glass centrifuge tube that had been well-cleaned and dried. After the centrifuge was quickly set to 5000 revolutions per minute for ten minutes, roughly half of the supernatant serum was removed using a Pasteur pipette and placed in a sterile, dry glass serology tube.

Isolated aortic rings:Performed according to Nicosia and Ottinett (1990) (11).
Biochemical measurements: serum insulin level, blood glucose measurements, lipid Profile, oxidative sress including serum MAD and Catalasemeasurements**.**Renal function tests;serum creatinine &uric acid measurements.

Statistical analysis: Data expressed as mean ± SEM. The one-way analysis of variance (ANOVA) was used, followed by the post hoc Tukey test to determine the statistical significance of the differences between the groups. A P-value <0.05 was considered significant.

Results

The results show that compared to normal rats, untreated diabetic rats had a much higher contractile response of the aorta (P<0.001). Compared to untreated diabetic rats, diabetic rats treated with glimepiride showed a substantial decrease (P<0.05) in the contractile response. Nevertheless, as demonstrated in Figure 1, diabetic animals administered glimepiride still exhibited a notably higher aortic response than normal rats (P<0.05).

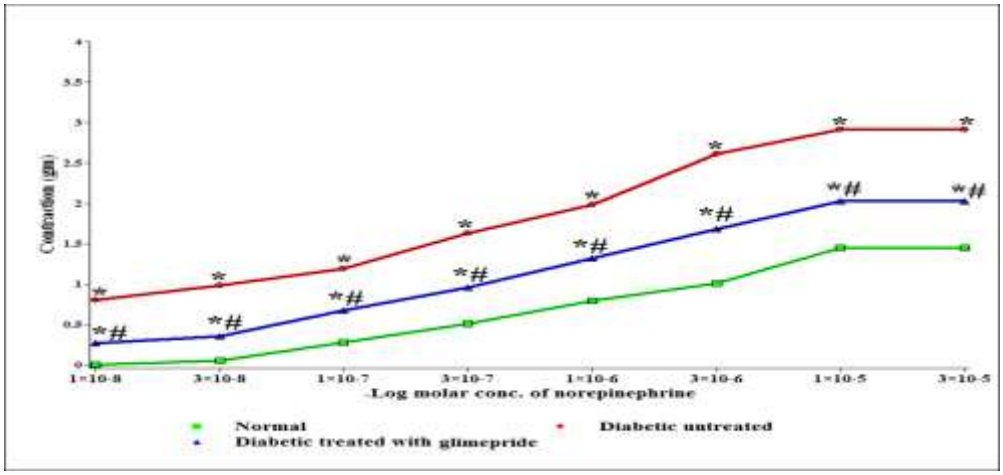

Figure (1):The impact of glimepiride on the contractile response of aorta isolatedfrom diabetic rats to noradrenaline.

Losartan significantly reduced the contractile response of the aorta in diabetic rats (P< 0.05) compared to untreated diabetic rats, according to the data. The aorta of normal rats showed no change, although there was a significant (P< 0.05) rise. The aortic contraction in diabetic rats was significantly reduced (P<0.001) after losartan and glimepiride were given to the rats. As seen in figure (2), there was no statistically significant difference (P>0.05) between this contraction and the one seen in normal rats.

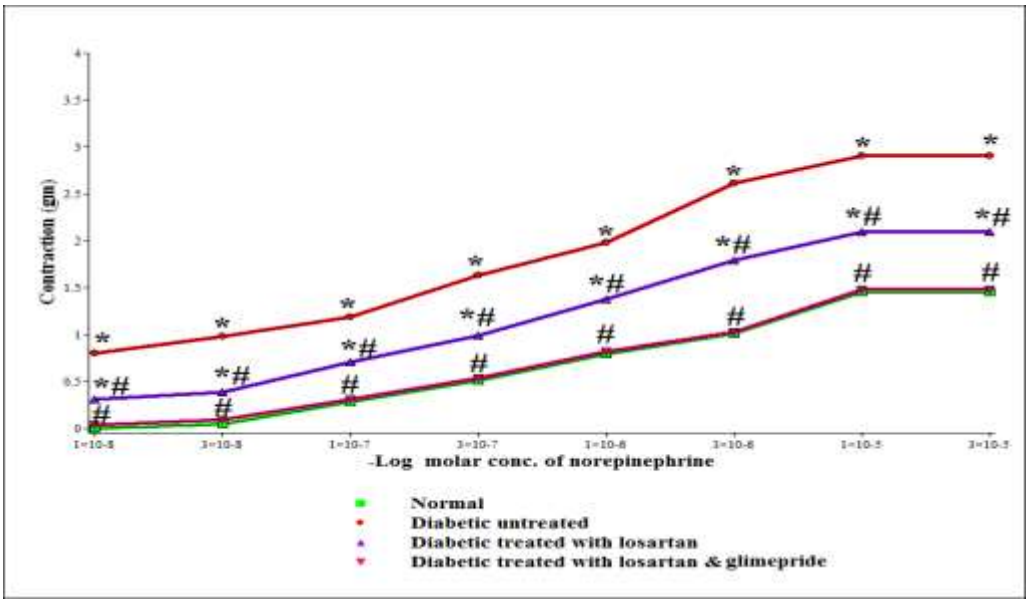

Figure (2): The impact of losartan on the contractile response of aortaisolated from diabetic rats to noradrenaline.

According to the results, atorvastatin significantly decreased the contractile response of the aorta in diabetes rats (P<0.05) when compared to untreated diabetic rats. The aorta of normal rats showed no change, although there was a significant (P<0.05) rise. Diabetic rats' aortic contractile response was

significantly reduced (P<0.01) after receiving atorvastatin and glimepiride. Figure 3 shows that this decrease was not substantially different from normal rats (P>0.05).

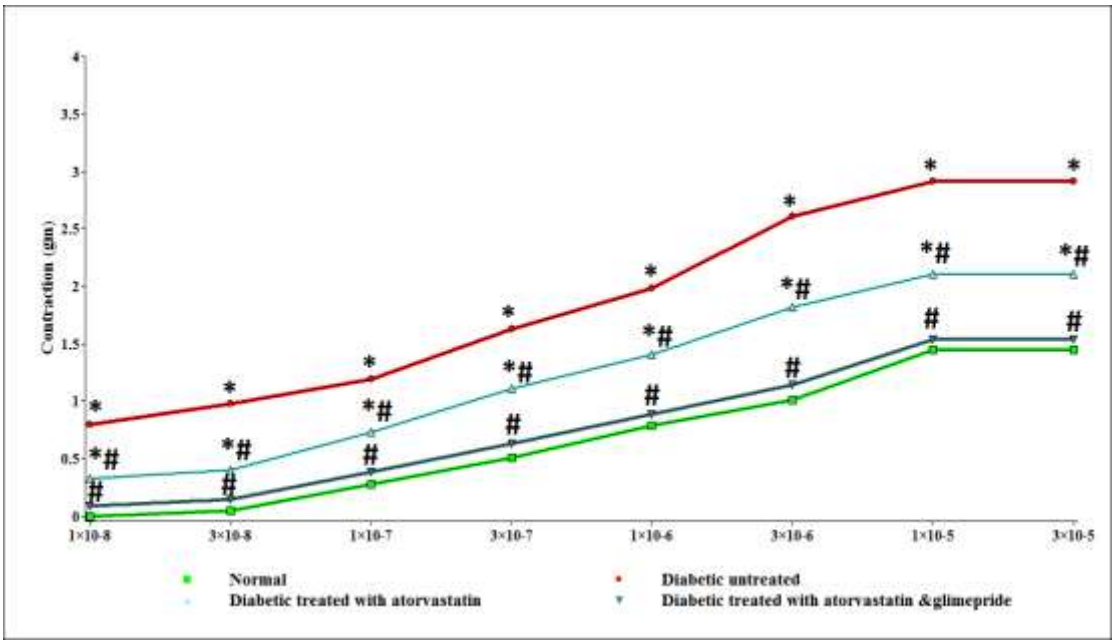

Figure (3):The impact of atorvastatin on the contractile response of aortaisolated from diabetic rats to noradrenaline.

The results indicate a significant decrease (P<0.001) in the relaxant response of the aorta in untreated diabetic rats compared to normal rats. However, the relaxant response significantly increased (P<0.01) in diabetic rats treated with glimepiride compared to untreated diabetic rats. Figure 4 shows that glimepiride-treated diabetic rats had a much lower relaxant response of the aorta than normal rats (P>0.05).

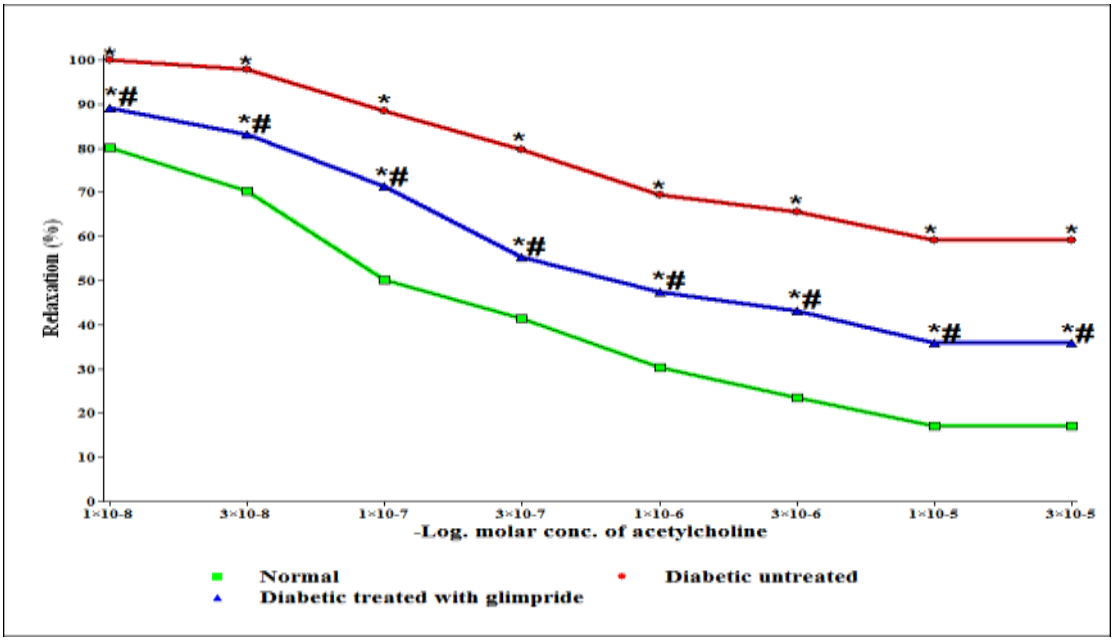

Figure (4): The impact of glimepiride administration on the relaxat response of aortaisolatedfrom diabetic rats to ACh.

Losartan considerably improved the relaxation response of the aorta in diabetic rats (P<0.01) as compared to untreated diabetic rats, according to the findings. But compared to normal rats, there was

still a significant (P< 0.05) drop. When losartan and glimepiride were given to diabetic rats, their aorta's relaxation response was significantly enhanced (P< 0.01). Figure 5 shows that this increment was not statistically different from normal rats (P> 0.05).

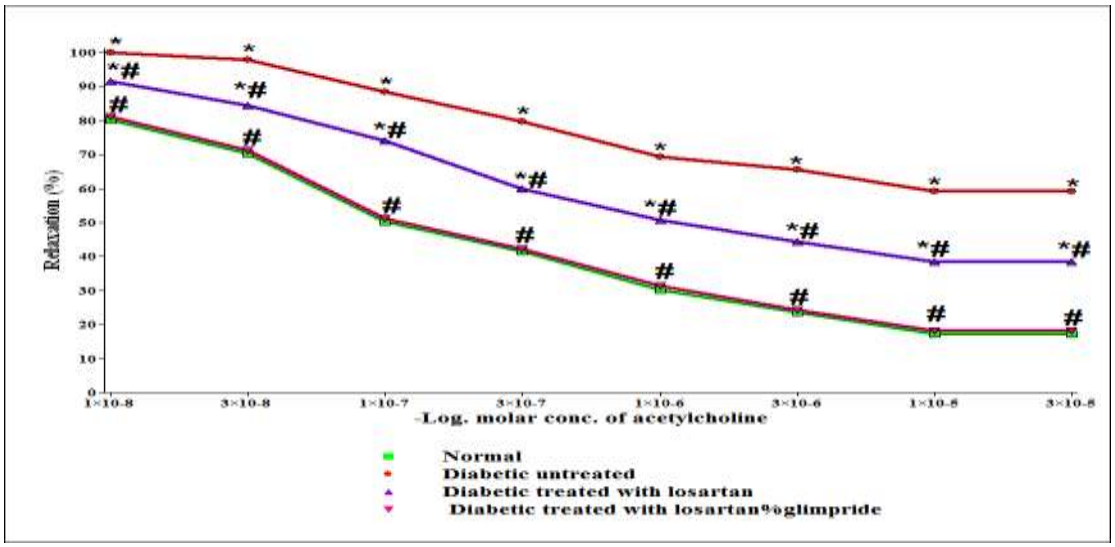

Figure (5): The impact of losartan on the relaxat response of aortaisolatedfrom diabetic rats to ACh.

In comparison to untreated diabetic rats, the results show that atorvastatin dramatically improved the relaxation response of the aorta in diabetic rats. The reduction was nevertheless significant (P<0.05) when compared to rats who did not have any abnormalities. When diabetic rats were given atorvastatin plus glimepiride, their aortic relaxation response was significantly enhanced (P<0.01). Compared to normal rats, there was no significant difference (P>0.05) in this rise, as shown in figure 6.

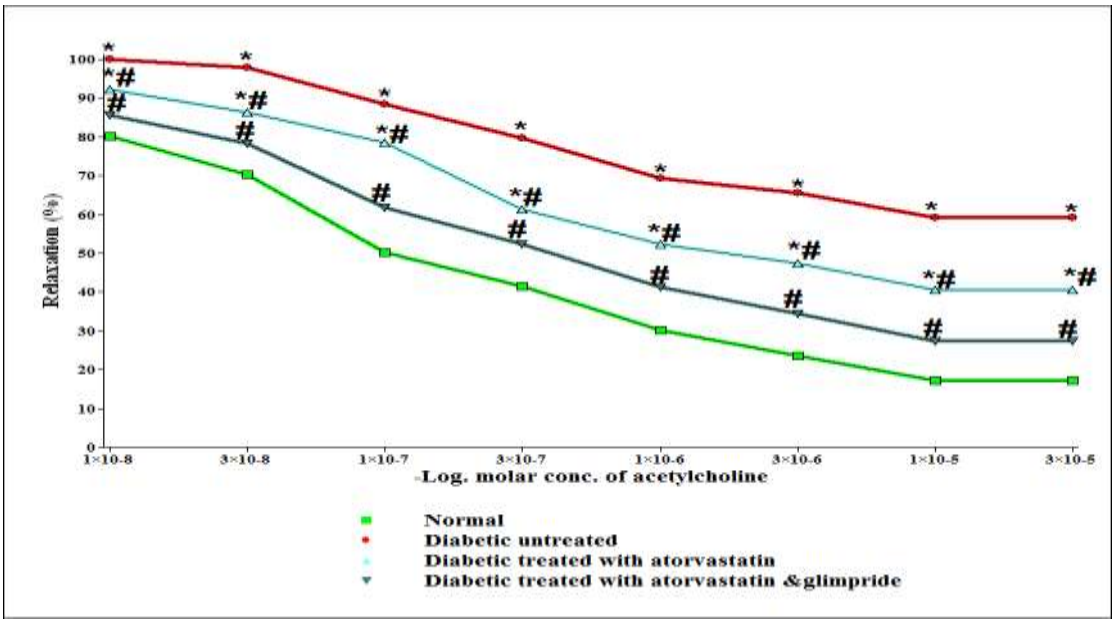

Figure (6): The impact of atorvastatin on the relaxat response of aortaisolatedfrom diabetic rats to ACh.

Biochemical assays

Effect of oral treatment with glimepiride, losartan, atorvastatinand combinations of glimepiridewithlosartanor atorvastatin, combinations oflosartan withatorvastatinon glucose

homeostasis, lipid profile, oxidative sress parameters and serum creatinine and uric acid levels in diabetic rats.

1. Glucose homeostasis: Fasting blood glucose (FBG), fasting serum insulin (FSI), (Table 1 (a, b).

Table (1):

a- FBG (mg/dl)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	85.53	277.31	180.14	200.41	204.23	170.31	175.27	168.17
± SEM	3.92	6.71	6.13	7.01	8.25	5.14	6.74	6.01
Pa		<0.001						
Pb			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc						<0.001	<0.001	<0.001
b-Fasting serum insulin (µU/ml)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	8.21	4.19	7.01	6.83	6.79	7.74	7.89	7.45
± SEM	1.14	1.02	0.47	0.17	1.34	0.49	0.12	0.74
Pa		<0.001						
Pb			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc						<0.001	<0.001	<0.001

Pa: Test of significance between control normal and control diabetic.
Pb: Test of significance between control diabetic group and all treated groups.
Pc: Test of significance between glimepiride group and combination treated groups.
- value: ≤ 0.05: Significant.

2- Total lipid profile: Total serum cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), serum triglycerides (TGs), high density lipoprotein-cholesterol (HDL-C) and atherogenic index. (Table 2 (a-e)).

Table (2):

a- Cholesterol (mg/dl)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	53.32	93.24	71.93	70.10	59.13	65.97	55.72	54.11
± SEM	2.91	3.18	2.94	3.01	2.10	3.02	2.15	2.37
Pa		<0.001						
Pb	-		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc	-					<0.001	<0.001	<0.001
b- LDL-C (mg/dl)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	9.01	38.09	10.29	11.14	8.86	8.04	8.96	8.73
± SEM	1.10	2.18	1.04	1.41	2.04	1.17	2.14	1.08
Pa		<0.001						
Pb	-		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc	-					<0.001	<0.001	<0.001
c- Triglycerides (mg/dl)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	23.71	84.01	67.43	70.57	60.91	66.01	25.86	22.93
± SEM	2.50	3.13	2.01	4.81	2.01	2.47	4.12	1.10
Pa		<0.001						
Pb			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc						<0.001	<0.001	<0.001
d- HDL-C (mg/dl)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin

Mean	41.81	20.01	31.02	30.18	29.81	38.93	40.74	40.15
± SEM	1.50	1.64	2.04	2.10	2.41	3.03	2.45	2.19
Pa		<0.001						
Pb	-		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc	-					<0.001	<0.001	<0.001
e-Atherogenic index	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	0.70	2.35	1.37	1.52	0.90	1.01	0.81	0.84
± SEM	0.10	0.11	0.01	0.12	0.28	0.17	0.19	2.12
Pa		<0.001						
Pb	-		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc	-					<0.001	<0.001	<0.001

Pa: Test of significance between control normal and control diabetic.
Pb: Test of significance between control diabetic group and all treated groups.
Pc: Test of significance between glimepiride group and combination treated groups.
- value: ≤ 0.05: Significant.

3. As regards TNFα, MDA. Table 3 (a, b)

a-TNFα (PG/ml)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	29.09	110.10	69.10	67.89	59.94	40.12	37.15	35.40
± SEM	1.12	7.37	5.85	5.76	4.02	3.89	2.27	2.29
Pa		<0.001						
Pb	-		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc	-					<0.001	<0.001	<0.001
b-MDA (mmol/ml)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	51.01	160.04	100.13	98.97	101.08	77.57	62.02	60.87
± SEM	2.74	6.29	4.58	4.24	6.01	4.04	4.14	4.20
Pa		<0.001						
Pb	-		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc	-					<0.001	<0.001	<0.001

Pa: Test of significance between control normal and control diabetic.
Pb: Test of significance between control diabetic group and all treated groups.
Pc: Test of significance between glimepiride group and combination treated groups.
- value: ≤ 0.05: Significant.

4. As regards catalase enzyme (u/ml). (Table3)

Catalase (U/ml)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	125.01	59.75	88.05	84.07	81.14	100.15	120.43	118.59
± SEM	2.63	6.69	4.07	6.13	6.94	4.22	4.13	8.63
Pa		<0.001						
Pb	-		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc	-					<0.001	<0.001	<0.001

Pa: Test of significance between control normal and control diabetic.
Pb: Test of significance between control diabetic group and all treated groups.
Pc: Test of significance between glimepiride group and combination treated groups.

- value: ≤ 0.05: Significant.

5.Kidney function tests*: Serum uric acid and creatinine levels.* (Table 4 a & b)

a- Uric acid (mg/dl)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride + atorvastatin	losartan + atorvastatin
Mean	0.80	2.82	1.05	1.11	1.03	0.95	0.89	0.91
± SEM	0.10	0.19	0.20	0.11	0.13	0.04	0.16	0.15
Pa		<0.001						
Pb			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc						<0.001	<0.001	<0.001
b-Creatinine (mg/dl)	Control Normal	Diabetic non treated	glimepiride	losartan	atorvastatin	glimepiride + losartan	glimepiride+ atorvastatin	losartan+ atorvastatin
Mean	0.72	1.90	1.02	1.04	1.01	0.80	0.77	0.75
± SEM	0.01	0.11	0.02	0.12	0.14	0.08	0.05	0.14
Pa		<0.001						
Pb			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pc						<0.001	<0.001	<0.001

Pa: Test of significance between control normal and control diabetic.
Pb: Test of significance between control diabetic group and all treated groups.
Pc: Test of significance between glimepiride group and combination treated groups.
- value: ≤ 0.05: Significant.

Discussion

We compared the effects of glimepiride, losartan& atorvastatin on kidney function , vascular reactivity , insulin level, oxidative stress parameters, lipid level, & blood glucose level in rats with diabetes induced by streptozotocin.

Constriction of the isolated rat aortic rings induced by norepinephrine (NE) was significantly greater in untreated diabetic rats as compared to healthy rats, according to the findings of the research. Untreated diabetic rats showed a significantly diminished aortic relaxation response to precontracted NE aortic ring preparations induced by acetylcholine (ACh) in comparison to normal rats.

This finding aligns with the results reported by **Desuki et al. (12)** that demonstrated a decline in vascular reactivity in cases with diabetes. Additionally, they observed that the aortic response to phenylephrine (PE) and KCl increased significantly, while it decreased significantly in response to ACh.In their study, Roghani et al. (13) observed a significant increase in aortic constriction caused by KCl and PE in diabetic rats in comparable to the control group. The observed outcome is attributed to compromised endothelial function, heightened calcium channel sensitivity, and a higher level of vasoconstrictive prostanoids resulting from elevated oxygen levels in diabetic rats.The observed variations could potentially be caused by factors such as the distinct experimental conditions employed in both studies, the specific agonist or type of artery investigated, the methods utilized in the vascular research, or the length of time of diabetes.(**14)**

The present research findings indicate that the contractile aorta response to norepinephrine significantly declined in diabetic rats treated with glimepiride, compared to untreated diabetic rats. Furthermore, diabetic rats treated with glimepiride exhibited a significantly improved Ach relaxation response to pre-contracted aortic ring preparations in the NE when compared to diabetic rats that were not treated with glimepiride.

The findings derived from the current study are consistent with those reported by **Vandana et al. (15).** Glimepiride therapy improved endothelium-dependent vascular relaxation in diabetic rats, according to the authors. These beneficial effects on the vasculature may be attributable to the drug's antioxidant properties, metabolic actions, & improvements in plasma lipids & fasting glycemia.

The aortic contraction caused by norepinephrine is considerably reduced in diabetic rats given losartan compared to diabetic rats untreated with losartan, according to the findings of the present research. Furthermore, the relaxing impact of acetylcholine on norepinephrine-precontracted aortic ring preparations was notably amplified in diabetic rats that received losartan compared to diabetic ratsthat did not receive any treatment.

However, this distinction wasn't statistically significant when compared to normal rats. However, the endothelial functions of the aorta of diabetic rats recovered through therapy with a combination of losartan and glimepiride.

According to a study by **Mostafa et al. (16),** the aorta of rats with diabetes demonstrated increased oxygen production, which was correlated with reduced eNOS expression. Losartan or L-carnitine treatment significantly increased NO availability by raising eNOS expression.

Endothelial dysfunction in rats with metabolic syndrome (MS), according to **Xiang et al. (17),** was distinguished by endothelium-dependent vasorelaxation that was depressed. The administration of losartan & pioglitazone in combination significantly reduced endothelial dysfunction in MS rats compared to either drug used alone. NO is a crucial regulator of vascular tone & blood pressure among many molecules derived from endothelium.

The findings derived from the current investigation are consistent with those reported by **Manpreet et al. (18),** that streptozotocin-treatedratsexhibited a significant rise in levelsof blood glucose due to a rise in ROS production caused by the disruption of a single-strand of DNA, which activates PARP & causes necrotic and apoptotic death of islets of Langerhans.

This study shows that compared to control rats, untreated diabetic rats had much lower serum insulin levels and much higher fasting blood glucose levels. Glimepiride significantly reduced fasting blood glucose and increased serum insulin levels in diabetic rats compared to those rats that did not get the drug. These findings corroborated those of **Mohamed et al. (19),** who showed that, compared to the control group, streptozotocin-induced a large increase in serum glucose and a marked decrease in serum insulin levels. Blood glucose levels dropped significantly and insulin levels rose significantly after glimepiride injection.

According to the findings of **Marwa et al. (20),** type II diabetes mellitus is distinguished by higherlevels of blood glucose & a decreased insulin sensitivity index; these effects are observed following a single i.p. injection of streptozotocin (50 mg/kg). Furthermore, after two weeks of daily dosing, the authors showed that diabetic rats induced with STZ had a marked decrease in serum glucose level when given glimepiride (0.5 mg/kg). Glimepiride significantly reduced glucose levels and increased insulin levels in diabetic rats, as shown by **Saleh and Maged (21).**

The current study demonstrates that diabetic rats treated with losartan had a significantly lower level of fasting blood glucose & a greater level of serum insulin compared to the untreated rats. Furthermore, our findings indicate that diabetic rats treated with a mixture of losartan & glimepiride had their fasting blood glucose & serum insulin levels repaired to normal.

In their study, **Nesren et al. (22)** demonstrated that when losartan, an AgII receptor blocker, was used in conjunction with an oral hypoglycemic agent, fasting blood glucose levels in cases with type 2 DM reduced significantly. This finding is consistent with the results stated by **Jin and Pan (23)** which suggest that administering losartan in diabetic cases with nephropathy at therapeutic doses (relatively high) significantly decreases fasting blood glucose levels. This effect is primarily attributed to a rise in insulin sensitivity. Furthermore, in the absence of insulin, there is a suggestion that the plasma

glucose-lowering activity of angiotensin receptor blockers may be accompanied by a rise in peripheral tissue glucose utilization and/or a decrease in hepatic gluconeogenesis **(24).**

While the precise mechanism underlying drug-induced hypoglycemia remains unknown, one hypothesis suggests that the elevation in bradykinin levels associated with the use of ACE inhibitors could potentially enhance insulin sensitivity **(25).**

In streptozotocin-diabetic rats, insulin deficiency is correlated with hypertriglyceridemia & hypercholesterolemia, according to **Goyal et al. (26)**. A cluster of risk factors for coronary disease, which involves hypertension, abdominal obesity, hyperinsulinemia, & insulin resistance, involves a diminished level of plasma HDL.

The findings of the current work indicate that diabetic rats exhibited elevated levels of total cholesterol, triglycerides, & LDL in their serum, while normal rats demonstrated a corresponding reduction in HDL concentrations. The administration of glimepiride effectively mitigated the impact of diabetes-induced changes in lipid levels.
In diabetic rats treated with losartan, the total levels of serum triglycerides, cholesterol,&low-density lipoproteindeclined significantly, whereas heigh-density lipoprotein rose significantly, according to the current study.

According to a study by **Nesren et al. (20),** diabetic cases who received captopril or losartan they suffered a significantly greater improvement in their lipid profile than those who received onlyoral hypoglycemic agents. The local renin-angiotensin system in skeletal muscles is disrupted, which causes the variance, which has been found to impact exercise performance & carbohydrate metabolism in this region.**(27).**

A significant long-term complication of diabetes mellitus is diabetic nephropathy. Microalbuminuria, which develops gradually to proteinuria, increased blood pressure, and impaired renal function, are clinical symptoms **(28).** Excessive glomerular buildup of extracellular matrix protein and subsequent mesangial enlargement are the main structural alterations in diabetic nephropathy. **(29).** There is a growing body of evidence indicating that patients diagnosed with DM exhibit elevated levels of lipoprotein oxidation. The pathogenesis of renal injury may be influenced by hyperlipidemia, which is also regarded as a risk factor for diabetic nephropath**y.(30)**
Nagy (31) documented that the amelioration of previous morphological changes in the kidneys of rats treated with glimepiride, as well as the improvement in blood urea and serum creatinine levels, may have been due to the restoration of renal function. The recovery observed can be attributed to the regeneration ability of the renal tubules since optimal metabolic control helps to slow the advancement of renal dysfunction related to diabetes.

Conclusion

RAAS blockers (losartan and enalapril) and atorvastatin amelioratedSZT-induced diabetic nephropathy, impairment of lipid profile, and endothelial dysfunction through antihypertensive, antioxidant and anti-inflammatory activities. They can protect against diabetic-induced renal and cardiovascular complications.RAAS blockers (losartan and enalapril) and atorvastatine synergize thehypoglycaemic effect of glimepiride, thus the dose of glimepiride may be reduced in combination therapy which leads to minimum side effects. However, further clinical researchis recommended to investigate the exact mechanism of action and the potential of combined therapy.

References:

1. **Choudhury, A. A., & Rajeswari, V. D. (2021).** Gestational diabetes mellitus-Awasametabolic and reproductive disorder. *Biomedicine & Pharmacotherapy*, *143*, 112183.

2. **Molehin, O. R., Adefegha, S. A., &Adeyanju, A. A. (2020).** Role of oxidative stress in the pathophysiology of type 2 diabetes and cardiovascular diseases. *Role of oxidative stress in pathophysiology of diseases*, 277-297.
3. **Zhang, Y., Murugesan, P., Huang, K. *et al.* (2020)**NADPH oxidases and oxidase crosstalk in cardiovascular diseases: novel therapeutic targets. *Nat Rev Cardiol* 17, 170–194.
4. **Yun, J.S. and Ko, S.H. (2021).**Current trends in epidemiology of cardiovascular disease and cardiovascular risk management in type 2 diabetes. Metabolism.;123:154838.
5. **Paget GE and Barnes JM. (1964):** Toxicity tests in Evaluation of drug activities and pharmacometrics. Ed, Laurance, D.R. and Bacharch, A.L. Acad press Massachusetts;135-166.
6. **Wszola, M., Klak, M., Kosowska, A., Tymicki, G., Berman, A., Adamiok-Ostrowska, A., ... & Kaminski, A. (2021).** Streptozotocin-induced diabetes in a mouse model (BALB/c) is not an effective model for research on transplantation procedures in the treatment of type 1 diabetes. *Biomedicines*, *9*(12), 1790.
7. **Elankani P., Reddy, G. D., Kabilan, N., Kumar, P., Ganesan, R.. Kumar, G. V. N. and Pitchaiah, D. (2020).**EVALUATION OF THE ANTI-DIABETIC ACTIVITY OF THE TRAN VIDHAI KUDINEER ON FRUCTOSE AND STREPTOZOTOCIN INDUCED DIABETIC RATS.INTERNATIONAL JOURNAL OF Pharmaceutical Sciences and Research.
8. **Biswas, S. K., Chatterjee, S., Laha, S., Pakira, V., Som, N. K., Saha, S., & Chakraborty, S. (2022).** Instrument-free single-step direct estimation of the plasma glucose level from one drop of blood using smartphone-interfaced analytics on a paper strip. *Lab on a Chip*, *22*(23), 4666-4679.
9. **Silva-Reis, R., Faustino-Rocha, A. I., Silva, J., Valada, A., Azevedo, T., Anjos, L., & Oliveira, P. A. (2021).** Studying and Analyzing Humane Endpoints in the Fructose-Fed and Streptozotocin-Injected Rat Model of Diabetes. *Animals*, *13*(8), 1397.
10. **Wszola, M., Klak, M., Kosowska, A., Tymicki, G., Berman, A., Adamiok-Ostrowska, A., & Kaminski, A. (2021).** Streptozotocin-induced diabetes in a mouse model (BALB/c) is not an effective model for research on transplantation procedures in the treatment of type 1 diabetes. *Biomedicines*, *9*(12), 1790.
11. **Nicosia, R. F. and Ottinetti, A. (1990)**. Growth of microvessels in serum-free matrix culture of rat aorta: a quantitative assay of angiogenesis in vitro. *Lab. Invest.* **63**, 115–122.
12. **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.
13. **Roghani M, Jalali-Nadoushan MR, Baluchnejadmojarad T, Vaezmahdavi 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,12(3):377-385.
14. **Fabiano E. Xavier, Ana Paula C. Davel, Luciana V. Rossoni, Dalton V. Vassallo (2003):** Time-dependent hyperreactivity to phenylephrine in aorta from untreated diabetic rats: role of prostanoids and calcium mobilization Vascular Pharmacology 40 : 67– 76.
15. **Vandana S. Nade, L. A. Kawale And K. M. Patel (2015):**Protective Effect of Sitagliptin and Rosuvastatin Combination on Vascular Endothelial Dysfunction in Type-2 Diabetes Indian Journal of Pharmaceutical Sciences 77(1):96-102.
16. **Mostafa Sleem, Ashraf Taye, Mohamed A. El-Moselhy, Safwat A. Mangoura (2014)**: Combination therapy with losartan and L -carnitine protects againstendothelial dysfunction of streptozotocin-induced diabetic ratsEuropean Journal of Pharmacology -744-10-17.
17. **Xiang Kong, Ming-zhe Ma, Li Qin, Yan Zhang, Xiao-yong Li1, Guo-dong Wang, Qing Su1 and Dao-you Zhang (2014):** Pioglitazone enhances the blood pressure-lowering effect of losartan via synergistic attenuation of angiotensin II-induced vasoconstriction. Journal of the Renin- Angiotensin-Aldosterone System. 15(3):259-70.

18. **Manpreet Kaur, Shilpi Sachdeva, Onkar Bedi, Tavleen Kaur, and Puneet Kumar (2015):**Combined effect of hydrogen sulphide donor and losartan in experimental diabetic nephropathy in ratsJ Diabetes MetabDisord; 14: 63.
19. **Mohamed I Saad, Maher A Kamel, Mervat Y Hanafi, Madiha H Helmy, Rowaida R Shehata(2014):** Effect of Sitagliptin and Glimepiride on Glucose Homeostasis and cAMP Levels in Peripheral Tissues of HFD/STZ Diabetic Rats American Journal of Biomedical Research, , Vol. 2, No. 3, 52-60.
20. **Marwa M.A. Khalaf, Gamal A. El Sherbiny, Hekma A. AbdEllatif, Afaf A. Ain-shoka, Mostafa E. El Sayed. (2012):** Comparative Effects of Glimepiride, Vanadyl Sulfate and Their Combination on Hypoglycemic Parameters and Oxidative Stress British Journal of Pharmacology and Toxicology 3(6): 278-288.
21. **Saleh N. Mwafy and Maged M. Yassin, (2011):** Antidiabetic Activity Evaluation of Glimepiride and Nerium oleander Extract on Insulin, Glucose Levels and Some Liver Enzymes Activities in Experimental Diabetic Rat Model. Pakistan Journal of Biological Sciences, 14: 984-990.
22. **Nesren Shaban Mohammed ,Esam Noori Al-Karwi, Ahmed Tariq Numan and Saad Abdul-Rehman Hussain(2010):** Effects of Low Doses of Captopril or Losartan in Improving Glycemic Control by Oral Hypoglycemic Agents in Type 2 DM Patients AJPS, Vol. 8, No.2.
23. **Jin H., Pan Y. (2007):** Angiotensin type-1 receptor blockade with losartan increases insulin sensitivity and improves glucose homeostasis in subjects with type2 diabetes and nephropathy. Nephrol Dial Transplant 22: 1943–1949.
24. **Yadav, N., Palkhede, J. D., & Kim, S. Y. (2021).** Anti-Glucotoxicity Effect of Phytoconstituents via Inhibiting MGO-AGEs Formation and Breaking MGO-AGEs. International Journal of Molecular Sciences, 24(8), 7672.
25. **Reghunath, S. R., Rashid, M., Chandran, V. P., Thunga, G., Shivashankar, K. N., & Acharya, L. D. (2021).** Factors contributing to the adverse drug reactions associated with the dipeptidyl peptidase-4 (DPP-4) inhibitors: A scoping review. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 102790.
26. **Goyal BR, Shraddha V. Bhadada and Mayur M. Patel. (2011):** 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
27. **Oyedepo, T. A., &Palai, S. (2021).** Herbal remedies, toxicity, and regulations. In Preparation of Phytopharmaceuticals for the Management of Disorders (pp. 89-127). Academic Press.
28. **Hassanin A, Malek HA (2014).** Effect of renin inhibition on adipokines in diabetic rats. Pak J Pharm Sci.;27(4):767–72.
29. **Xu D, Liu J, Ji C, Zhou L, Hong Guo (2007):** Effects of telmisartan on hypertensive patients with dyslipidemia and insulin resistance. Journal of Geriatric Cardiology Vol 4 No 3 149-152.
30. **Roberto I. Mota, Samuel E. Morgan & Edward M. Bahnson. (2020).** Diabetic Vasculopathy: Macro and Microvascular Injury. Current Pathobiology Reports 8:1–14.
31. **Nagy M.A.(2015):** Antidiabetic, antihyperlipidemic and histopathological analysis of *Panax quinquefolium* extract on alloxan induced diabetic rats *An Indian Journal* BCAIJ, 9(2), [041-050].