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The Renin Inhibitor Aliskiren mitigates Diabetes mellitus induced Vascular Impairment of rats

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Abstract

Background: The most prevalent kind of diabetes is type 2 diabetes mellitus (T2DM).Vascular impairment is one of the numerous problems that frequently accompany diabetes mellitus.Renin inhibitors like Aliskiren have been shown to effectively protect organs through a variety of methods. **Objective**: to illustrate aliskiren's vascular-protective effects in diabetic rats and any possible underlying processes at play. **Material and methods**: Thirty male albino rats were split into three groups: diabetic, diabetic+aliskiren, and control (10/group). Serum glucose, insulin, HOMA-IR index, glycosylated Hb A1c, cholesterol, triglycerides, renin activity, aortic MDA, aortic SOD, aortic TNF-α, aortic IL-6, aortic IL-10, aortic NF-kB gene expression, and aortic ENOS gene expression were all evaluated. Additionally, aortic histology and immunohistochemical analyses were performed. **Results**: In contrast to the control group, the diabetic group's aortic SOD, aortic IL-10, and aortic gene expression of ENOS were significantly lower, while the measured serum glucose, insulin, HOMA-IR index, serum glycosylated Hb A1c, serum cholesterol, serum triglyceride, serum renin activity, aortic MDA, aortic TNF-α, aortic IL-6, and aortic gene expression of NF-kB were all significantly higher in the diabetic group. Additionally, as compared to the control group, the diabetic group's aortic ENOS was significantly downregulated and their aortic NF-kB immunoreaction was elevated. Aliskiren considerably reduced the vascular alterations brought on by diabetes. **Conclusion**: By blocking renin activity and exhibiting hypoglycemic, lipid-lowering, anti-inflammatory, and antioxidant effects in addition to upregulating aortic ENOS, aliskiren guards against diabetes-induced vascular damage.

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Introduction

Diabetes mellitus (DM) is a worldwide health concern that is expanding faster than anticipated. According to the most recent International Diabetes Federation (IDF) Diabetes Atlas, 10.5% of people worldwide have diabetes, and 44.7% of adults do not have a diagnosis.By 2045, one in eight individuals, or 783 million people, would have diabetes, according to IDF projections. This would more than double the anticipated 20% population growth, or a 46% increase over the same time period [1].

More than 80% of instances of diabetes are type 2 diabetes, the non-insulin-dependent form of the disease. It frequently coexists with obesity and can proceed quickly, resulting in a number of diabetic complications. Type 2 diabetes is characterized by variable degrees of elevated blood glucose and insulin resistance (IR), which is the primary cause of the disease and the foundation for the development of cardiovascular problems in diabetic patients, according to studies [2].

The main characteristics of type 2 diabetes are insulin resistance and elevated blood glucose levels, which eventually result in crippling vascular system diseases [3]

The primary cause of death for diabetics is vascular problems. Antihyperglycemic medications' beneficial vascular benefits during type 2 diabetes are still insufficient. There is an urgent need for research on treatment approaches that can both lower blood sugar and protect blood vessels. There is currently no medication that targets vascular problems in diabetes directly [4]

Vascular problems and type 2 diabetes are becoming more common. Endothelial dysfunction has been found in pre-T2DM and the early phases of T2DM, according to clinical and experimental research [5]

Dyslipidemia, a typical finding in advanced type 2 diabetes ,is a significant issue to consider in diabetic vascular complications because it impairs arterial integrity and causes early atherosclerotic disease [6].

One of the main causes of vascular dysfunction in diabetes has been identified as vascular ROS generation and the oxidative stress it causes [7]

It has been proposed that diabetes impairs endothelial function, which underpins both micro- and macro-vascular problems, and that both conduit and resistance arteries, including the aorta, are dysfunctional. Although the underlying causes of these difficulties are complicated, oxidative stress and ROS generation are thought to be significant factors. In the aorta of diabetic rats, lipid peroxidation and ROS overproduction have been shown. This has resulted in the generation of chemicals that damage cell membranes and the glycosylation of proteins, both of which have been linked to vascular dysfunctions in diabetes [8].

Furthermore, In T2D, insulin failure triggers endothelial dysfunction, inflammatory reactions, and the production of ROS, which in turn triggers NF-kB. Insulin signaling, mitochondrial structure, cellular redox status, and malfunction are all significantly impacted by NF-kB.NF-kB is then transported to the nucleus by this mechanism, which also triggers the transcription of inflammatory cytokines [9].

Diabetes-induced oxygen radicals are a major contributor to the pathophysiology of cardiovascular disease because they hinder the synthesis of endothelial nitric acid. By producing NO and synthesizing bioactive chemicals that

maintain vascular homeostasis, vascular endothelium plays a critical role in regulating the tone and shape of the underlying vascular smooth muscle cells. For several of these endothelial functions, endothelial nitric oxide synthase (ENOS) is crucial. Vascular problems may arise as a result of direct or indirect inhibition of ENOS. Low levels of ENOS protein in the vascular endothelium of diabetic hearts have demonstrated the detrimental effects of diabetes on ENOS expression or activity. In the rat aorta, diabetes similarly decreased the expression of aortic ENOS [10].

Diabetes mellitus complication is significantly influenced by dysregulation of the renin-angiotensin-aldosterone system (RAAS). However, the majority of RAAS blockers donot fully prevent or reverse the consequences of diabetes. This may be because the negative feedback loop linked to decreased angiotensin II activation stimulates renin. Therefore, it seems more logical to use direct renin inhibition to completely and effectively block RAAS activity without causing a rebound increase in plasma renin activity (PRA)[11].

Aliskiren is the first medication in a novel class of substances called renin inhibitors, which lower PRA by directly inhibiting renin [12]**.** Effective organ protection associated with an anti-hypertensive impact was demonstrated by Aliskirentherapy. It has been noted that patients receiving RAAS inhibitors had a decreased incidence of diabetes mellitus. Numerous mechanisms have been proposed, including enhanced insulin signaling pathways, pancreatic beta cell release of insulin, and adipocytokineregulation in adipose tissues [11].

In addition to its ability to decrease blood pressure, aliskiren has recently been shown to have renoprotective, cardioprotective, anti-atherosclerotic, and antioxidant properties in animal models[13]. Additionally, in an animal model of atherosclerosis, aliskiren increases ENOS and enhances NO bioavailability [14]. Aliskiren has been shown to be able to significantly reduce inflammation[15]

In this work, we examined the potential underlying mechanisms and the vascular protective effect of aliskiren on diabetic vascular problems in type 2 diabetic rats.

Materials and methods

After obtaining the necessary approvals from MenoufiaUniversity, Egypt, Faculty of Medicine's Research Ethics Committee, IRB No. 5/2025PHYS12, thirty adult albino male rats weighing 100–150 g with age between 2 to 3 months purchased were used in the study. The rats were purchased from a local animal providing facility .ARRIVE standards was followed. The rats were housed in 80 × 40 × 30 cm wire mesh cages. After being acclimated to a consistent environment for two weeks, all animals were given regular access to food and water during the study period.

The rats were divided into three groups, 10 each:

1. Control group: For eight weeks, the rats were given ordinary chow along with a single intraperitoneal (i.p.) injection of 1 milliliter of citrate buffer which is the vehicle of STZ and 1 milliliter of normal saline orally which is the vehicle of the protective drug once a day.
2. Diabetic group: Rats were given free access to a high-fat diet for two weeks, consisting of 58% fat, 25% protein, and 17% carbohydrates as a percentage of total kcal. After 12 hours of fasting,

they received a single i.p. injection of STZ at a dose of 35 mg/kg BW dissolved in citrate buffer. In order to avoid hypoglycemia, 5 ml of a 5% glucose solution was then administered orally to each rat. One touch glucose strips and a glucometer (ACCU-CHEK) were used to measure the fasting blood glucose level by tail prick 72 hours after the STZ injection. Rats with blood sugar levels above 200 mg/dL were chosen for the investigation because they were deemed to have diabetes (Srinivasan et al., 2005).For eight weeks, the rats in this group were given free access to a high-fat diet and were given 1 milliliter of normal saline orally which is the vehicle of the protective drugonce a day[16].

3. Diabetic -Aliskiren -treated group (Diabetic+Aliskiren): As in the diabetic group, DM was induced. They received concurrent treatment for eight weeks with oral gavage of 10 mg/kg aliskiren (tekturnatablets, Novartis Egypt company; aliskiren 150 mg) dissolved in normal saline [17,14].

After the eight weeks blood samples were taken from the retro-orbital venous plexus of rats. Rats were subsequently sacrificed by cervical elongation and dislocation after being anesthetized. The thorax was opened and part of thoracic aorta was dissected. For histological and immunohistochemical evaluation, a portion of the aorta was preserved in 10% formalin saline. The remaining portion was utilized for RT-PCR and biochemical tests.

Blood sampling

Fasting blood samples were collected. Four milliliters of blood were extracted and divided equally between two tubes. The first tube was centrifuged for 10 minutes at 4000 revolutions per minute (rpm).Until it was needed for additional analysis, the serum was stored frozen at -20 °C.The calculation of glycosylated hemoglobin (Hb A1 c) was carried out after the blood from the second tube was transferred into an EDTA tube.

Calculation of HOMA-IR index

HOMA-IR index= fasting serum insulin (µU/mL)X fasting serum glucose (FSG) mg/dL/405[18]

Biochemical analysis

Colorimetric kits were used to assess fasting serum glucose (Diamond Diagnostic, Egypt) and serum HbA1c (StanbioGlycohemoglobin, Egypt).Following serum collection and freezing at -80°C, colorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt) were used to assess the serum's cholesterol and triglycerides (TG).The levels of plasma renin activity (Cat.: MBS041519, MyBioSource, Sandiego, CA, USA) and serum insulin (DRG Instruments GmbH, Marburg, Germany) were determined using the proper rat ELISA kits.

Tissue Homogenate Preparation

The weighted aortic tissues were individually homogenized. Then the crude tissue homogenate was centrifuged in an ice-cold centrifuge for 15 minutes. The supernatant was then collected and stored at -80°C for the test.

Aortic TNF-α (Cat.: MBS2507393, MyBioSource, Sandiego, CA, USA), aortic IL-6 (Cat.: MBS269892, MyBioSource, Sandiego, CA, USA), and aortic IL-10 (IL-10: ERI3010-1, Assaypro LLC, Saint Charles, Missouri, USA) were quantified using the ELISA Kit in accordance with the manufacturer's instructions.Calorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt) were used to test aortic MDA and superoxide

dismutase (SOD) in accordance with the manufacturer's instructions.

Quantitative RT-PCR (qRT-PCR)

A single piece of aorta tissue was taken from each rat and placed in a falcon tube, where it was kept at -80 °C for RNA extraction and the expression of the ENOS and NF-kBgenes.A 7500 real-time PCR machine (Applied Biosystems, CA, United States) was used to identify ENOS and NF-kB.A direct-zol RNA miniprep kit (Cat. No. R2051; Zymo Research, USA) was used to extract RNA from aortic cells. Then, using the QuantiTect Reverse Transcription Kit (205311; Qiagen, Applied Biosystems, USA), complementary deoxyribonucleic acid (DNA) was synthesized as the first step of PCR. Finally, the second PCR step (the real-time PCR step) was performed.

The following primers were used for the ENOS gene:

Forward,5′-CGA GAT ATC TTC AGT CCC AAG C-3,

Reverse 5′-GTG GAT TTG CTG CTC TCT AGG-3′.

The following primers were used for the NF-kBgene:

Forward,TCGACCTCCACCGGATCT

Reverse. GAGCAGTCATGTCCTTGG

The actin served as an endogenous control.Ten microliters of SYBR Green (2× QuantiTect PCR Master Mix), three microliters of cDNA, one microliter of forward primer, one microliter of reverse primer, and five milliliters of RNase-free water were used in each PCR reaction, which was carried out in a final volume of 20 microlitersThe data was processed using the Applied Biosystems 7500 software version 2.0.1.Gene expression was measured relative to one another using the comparative Ct technique.The amplification plot and melting curve of the ENOS and NF-kBgenes were conducted.

Histopathological Method

For histopathological studies, Aortic tissue sections were fixed at 10% formalin. Sections were prepared at 4 μm thickness and stained with Haematoxylin& Eosin.

For immunohistochemical studies, the Aortic paraffin sections (4 μm) were stained by ENOS1:1000, mouse monoclonal,Abcam ab76198), and NF-kB (monoclonal, dilution 1:200, Abcam).

Statistical analysis

Following data collection and analysis, they were found to satisfy the parametric assumptions based on the results of the Shapiro-Wilk test.As a result, one-way ANOVA and post hoc Bonferroni's tests were applied to the data.The data was displayed using the mean ± standard deviation (SD).Significance was considered to exist when the p value was 0.05 or less.The data was analyzed using Graph-Pad Prism software (version 9.3.1, San Diego, CA, USA).

Results

In contrast to the control group, the diabetic group's aortic SOD, aortic IL-10, and aortic gene expression of ENOS were significantly lower, while the measured serum glucose, insulin, HOMA-IR index, serum glycosylated Hb A1c, serum cholesterol, serum triglyceride, serum renin activity, aortic MDA, aortic TNF-α, aortic IL-6, and aortic gene expression of NF-kB were all dramatically elevated in the diabetic group. The Diabetic+Aliskiren had substantially higher levels

of aortic SOD, aortic IL-10, and aortic gene expression of ENOS than the Diabetic group, but significantly lower levels of serum glucose, serum insulin, HOMA-IR index, serum glycosylated Hb A1c, serum cholesterol, serum triglyceride, serum renin activity, aortic MDA, aortic TNF-α, aortic IL-6, and aortic gene expression of NF-kB. Table (1).

Table (1): The measured serum glucose, serum insulin, HOMA-IR index, serum glycosylated Hb $A1_c$, serum cholesterol, serum triglyceride, aortic MDA, aortic SOD, aortic TNF-α, aortic IL-6, aortic IL-10, aorticENOS gene expression and aorticNF-kB gene expression in all studied groups

	Control group	**Diabetic group**	**Diabetic+Aliskiren group**
Serum Glucose (mg/dl)	80.5±3.1	330.5±6.5*	240.8±7.5*#
Serum Insulin (µU/mL)	5.89±0.9	29.6±1.3*	21.8±1.2*#
HOMA-IR index	1.23±0.08	25.7±2.12*	13.25±2.91*#
Serum glycosylated Hb $A1_c$(% of normal Hb)	2.12±0.25	13.8±1.22*	8.9±0.18*#
Serum Cholesterol (mg/dL)	70.5±2.11	325.8±6.9*	270.9±7.8*#
Serum Triglyceride (mg/dL)	45.8±3.11	180.9±2.89*	139.5±2.12*#
Serum Renin (pg/ml)	29.18±3.89	110.89± 6.39*	38± 3.98*#
Aortic MDA (nmol/ gm. Tissue)	7.18±1.2	20.15± 1.03*	14.98± 1.11*#
Aortic SOD (U/gm. Tissue)	2.15±0.09	0.77±0.12*	1.33±0.18*#
Aortic TNF-α (pg/ml)	90.2±3.8	211.5±4.15*	166.8±3.14*#
Aortic IL-6 (pg/mL)	130.5±2.16	290.9±3.2*	211.8±3.35*#
Aortic IL-10 (ng/mL)	7.15±1.01	4.02±0.11*	5.9±0.18*#
AorticENOS gene expression	1	0.39±0.08*	0.61±0.11*#
AorticNF-kB gene expression	1	3.86±0.3*	1.87±0.12*#

* Significant compared with control, # Significant compared with Diabetic.

Histological results:

Hematoxylin and eosin (H&E):

Histological sections of the aorta (A) control group showed normal histological structure of the aota: tunica intima ,tunica media and tunica adventitia. (B) Diabetic group showing endothelial defect in the tunica intima and inflammation in the tunica adventitia.(C) Diabetic+Aliskiren group showing apparent restoration of the normal histology of the aorta . x400 magnification. (Fig. 1: A-C)

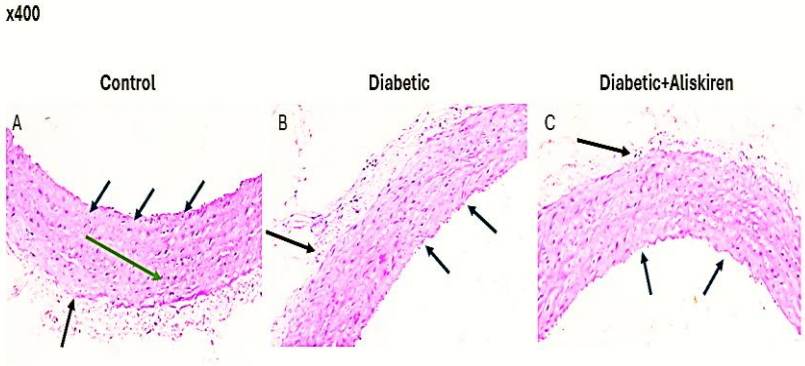

Fig. 1: Representative H&E histological sections of the aorta of (A) control showed normal histological structure of the three tunica of the aorta: tunica intima (blue arrows), tunica media (double headed arrow) and tunica adventitia (black arrow). (B) Diabetic showing enothelial defect in the tunica intima (blue arrows), also there is inflammation in the tunica adventitia (black arrow). (C) Diabetic+Aliskirengroup showing apparent restoration of the normal histology of the aorta in the tunica intima (blue arrow) and tunica adventitia (blue arrow).×400 magnification.

Immunohistochemical results

When compared to the control, the diabetic group's percentage area of aortic NF-kBimmunoreaction increased significantly (77.4 ±0.11 vs. 6.9.5±0.13, respectively, p<0.05).While this proportion was still higher than the control, it was dramatically lower in the Diabetic+Aliskiren group compared to the Diabetic (19.6±0.05 vs. 77.4±0.11, respectively, p<0.05).(Fig. 2: A-D)

When compared to the control, the diabetic group's percentage area of aortic ENOSimmunoreactiondecreased significantly (15.8±0.15 vs. 50.6±0.03, p<0.05).This proportion was higher in the Diabetic+Aliskiren group than in the Diabetic group (29.2.4±0.16 vs. 15.8±0.15, respectively, p<0.05), but it was still lower than in the control.(Fig. 2: E-H).

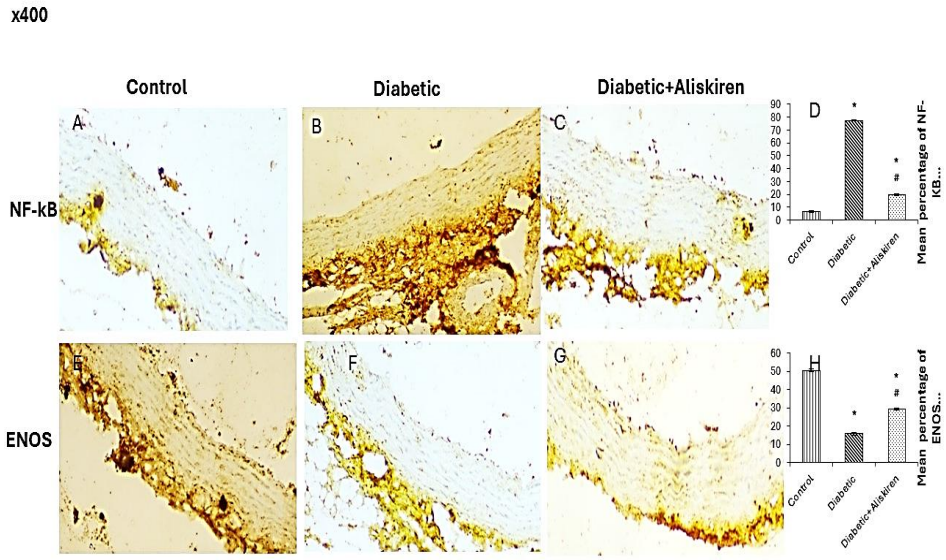

Fig (2): Representative micrographs of the different experimental groups showing a substantial upgrading of the NF-kB(A-D) immunoreaction in the Diabetic group and a dramatically decline in the Diabetic+Aliskiren group, however there is a significant downregulation of the ENOS (E-H) in the Diabetic group, but there is a substantialincrease in the Diabetic+Aliskiren group ×400 magnification

Discussion

Using a low dose of STZ and an HFD, T2DM was successfully induced.This was demonstrated in our study by the significantly worsened glycemic state and higher HOMA-IR of the diabetic group in comparison to the control group, which is consistent with other research[1].

Hyperlipidemia, a key characteristic of type 2 diabetes and one of the most prevalent aspects of DM in experimental rats, was seen in the results of the diabetic group.It is distinguished by elevated TG and cholesterol levels.According to earlier research, hyperlipidemia is caused by an excess of fat being mobilized from adipose tissue as a result of blood glucose being underutilized[1].

In keeping with earlier research, Aliskirinsignificantly improved the glycemic state and reduced the lipid profile and HOMA-IR of the Diabetic+Aliskiren group in comparison to the diabetic group [11].

Chou et al., 2011showed that Aliskiren prevents and reduces hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and hypercholesterolemia and ameliorated insulin resistance in rats fed fructose[19].

In animal models of obesity and type 2 diabetes, aliskirenincreases insulin sensitivity and guards

against pancreatic damage.Inhibiting the synthesis of Ang II and downregulatingthe local RAS in pancreatic islet and adipose tissue are two possible ways whereby direct renin inhibitors prevent and restore insulin resistance.Additionally, because of its vasoconstrictor properties, Ang II may cause the development of insulin resistance.Additionally, in fructose hypertensive rats, blocking the RAS with chronic ACE inhibition or ARB lowers oxidative stress and restores NO production, which can improve insulin signaling and reduce resistance in the skeletal muscle and pancreas [19].The hypolipidemic impact of aliskiren is attributed todownregulation of local RAS in adipose and liver tissue[19].

Aliskiren'scapacity to maintain hemodynamic alterations and reduce oxidative stress, inflammatory, and apoptotic indicators may be linked to its hypoglycemic effect[13].

In this study, we looked into how administering Aliskiren affected oxidative stress levels and the macro-vasculature complication of diabetic-induced aortic tissue damage. Our histological findings showed that STZ-induced diabetes exacerbated aortic tissue damage, which is consistent with other research[8].

Aliskiren treatment dramatically decreased the pathological aortic damage caused by diabetes. This result was followed by a decrease in lipid peroxidation markerMDA and an increase in the antioxidant enzyme level SOD in the aorta tissue of diabetic rats.

In this investigation, we discovered that the diabetic rat aorta developed vascular structural and pathological anomalies.One of the main causes of diabetic vascular system difficulties is the development of oxidative stress, and it has been proposed that hyperglycemia plays a crucial role in mediating the impact of oxidative stress on vascular integrity deterioration[8].

This was consistent with a prior study that showed much higher MDA and lower SOD in the aortic tissue of diabetic rats when compared to control [8].

Low-dose STZ produces free radicals that partially degenerate β-cells, while HFD leads to obesity and hyperinsulinemia.It is commonly recognized that oxidative free radicals are produced when a high-fat diet is combined with STZ[20].

Numerous studies have also shown a connection between oxidative stress and the RAAS[21].These findings imply that aliskerin's renin inhibition offered defense against the harm caused by oxidative stress [22].

Administration of Aliskirensignificantly reduced oxidative stress, which is consistent with other research[14], Whoclaimed that aliskiren'scapacity to raise antioxidant levels and upregulateNrf2 gene expression was responsible for its antioxidant effects[14].

NF-κBactivation is one of the primary drivers of ROS generation and, consequently, structural and functional alterations in the vasculature in the context of hyperglycemia.However, one of the processes frequently linked to oxidative stress is inflammation [8].NF-kB overexpression is caused by hyperglycemia. Activated NF-kBtravels to the nucleus to regulate the genes that produce proinflammatory cytokines [23].

In line with earlier research, our study demonstrated this by significantly increasing proinflammatory cytokines in aortic homogenate and aortic NF-kBgene expression and immunoreaction while significantly lowering the

anti-inflammatory mediator IL-10 in aortic homogenate of diabetic group compared to control[8].

Aliskirensignificantly increased IL-10 levels while significantly decreasing aortic inflammatory cytokines and aortic NF-kBgene expression and immunoreaction incomparison to the diabetes group.Aliskiren's anti-inflammatory effects are consistent with earlier research[22].

The NF-KB signaling pathway was activated by Ag II. This activation involves a significant signal transduction system that controls the expression of genes linked to inflammation.Numerous cytokines have their expression controlled by NF-kB [22].

Aliskiren'scapacity to suppress renin is thought to be the cause of its anti-inflammatory effects, as evidenced by the fact that it reduced plasma renin activity as compared to the diabetic group.The inflammatory reaction may be made worse by renin.As a result, blocking renin offers a desirable way to stop the RAAS at its source[15].Additionally, our study showed that Aliskirenhas an anti-inflammatory effect by raising IL-10 levels, which is consistent with other research[15].

In accordance with earlier research, the diabetic group showed higher renin activity than the control group, although aliskirinsignificantly reduced renin activity relative to the diabetes group [14].

The process that limits the rate at which angiotensin II (Ang II) is produced is renin.Therefore, Ang II levels are decreased by blocking this phase.ROSproduction, adhesion molecule upregulation, cytokine and chemokine release, and the start of NF-kB activation have all been linked to Ang II-induced organ damage [24].

According to a number of studies, endothelial cells produce nitric oxide (NO), one of the most significant vasodilators.Endothelial cells constitutively express ENOS. In accordance with earlier research, the diabetic group's aortic ENOS gene expression and aortic ENOS immunoreaction were dramaticallydownregulatedas compared to the control.[25]

According to Felaco et al. (2001), diabetic rat hearts have lower levels of ENOS protein in their arterial walls than do the hearts of healthy control rats.[26]

In diabetes mellitus, a nitric oxide function impairment appears extremely early.The reduction of ENOS protein in endothelial cells may be one factor contributing to the link between diabetes mellitus and atherosclerotic cardiovascular disease.[26]

In contrast to the diabetic group, aliskirensignificantly increased aortic ENOS immunoreaction and gene expression. This is consistent with a prior study that found that adenine significantly reduced ENOS expression in kidney tissues and that aliskiren administration significantly increased ENOS.[14]. Further studied with different doses and duration of treatment with aliskiren are required to clarify aliskirin effect on diabetes mellitus

Conclusion

By blocking renin activity and exhibiting hypoglycemic, lipid-lowering, anti-inflammatory, and antioxidant effects in addition to upregulatingENOS, aliskirenguards against diabetes-induced vascular damage.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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