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Original Article

Role of Brain Ang (1-7) with Combination Therapy of Aliskerin in Control of Diabetic Nephropathy

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ABSTRACT

Objectives: Angiotensin (1-7) system has been recognizing as physiologically major content of the renin-angiotensin system. It exhibited that Diabetic Nephropathy (DN), which is prevalent causes of end-stage renal disease, reduces Ang (1-7) peripheral activity. RAS activity in the PNS is controlled by RAS in the brain. The goal of this research is to see whether cerebral angiotensin (1-7) has a role in chronic diabetic kidney disease in wistar rats. **Materials and Methods:** Single dosage of streptozotocin 35 mg/kg i.p causes Diabetes Mellitus (DM). 2 doses of aliskerin and Ang (1-7) via a various route. After that there were testing of the samples. Commercially available kits were used to determine biochemical parameters linked to DN. **Results:** When streptozotocin was given to diabetic rats for 10 weeks, the mice displayed higher serum creatinine, blood urea as well as protein in urine, as well as a decreased amount of serum nitrite. A 2-week course of intracerebral aliskerin (100 nmol/day) and Ang (1-7) treatment decreased such changes also elevated serum nitrite in rats with DN, but only in conjunction with Ang (1-7) (4.8 g/day) separately or in combinations. **Conclusion:** The findings of current research imply that brain Ang (1-7) is vital in RAS peripheral activity modulation in diabetic nephropathy, which might be attributed to Ang II peripheral activity and reduced central sympathetic outflow.

Keywords: Angiotensin (1-7), Diabetic nephropathy, End-Stage Renal Disease (ESRD), Aliskerin, Peripheral activity, Renin angiotensin system.

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INTRODUCTION

One of the world's most debilitating diseases, Diabetes Mellitus (DM) affects millions of individuals. Insulin resistance or insulin insufficiency are the hallmarks of Diabetes Mellitus (DM), which results in increase in glucose concentration and changes in glucose, lipid and metabolism of protein.[1] Cardiomyopathy, retinopathy, neuropathy and nephropathy are long-term complications of diabetes. One of the most prevalent causes of kidney loss in diabetic people, Diabetic Nephropathy (DN) has become an international public health problem.

A sustained rise in albumin levels in the urine, an increase in the Glomerular Filtration Rate (GFR), Glial hypotrophy, tubule interstitial fibromatosis, mesangial hypertrophy as well as podocyte loss are all signs of DN in addition to increased extracellular matrix protein deposition. Blood Urea Nitrogen (BUN) as well as serum creatinine levels skyrocket when DN is followed by dyslipidemia. Although the actual origin of DN is unclear, hyperglycemia is caused by a variety of factors including hyper filtration, renal damage, advanced glycation end products and cytokine activation. The RAS seems to play a vital part in the pathophysiology of DN, according to a large body of research. During the DN, the peripheral RAAS becomes overactive. At the time of DN, the leading effector octapeptide Angiotensin II (Ang II) is over expressed, resulting in hyper filtration and increased intraglomerular pressure, as well as stimulation of mesangial cell proliferation. In clinical trials, angiotensin-converting enzyme inhibitors as well as type 1 angiotensin ii receptor antagonists have shown to delay the course of DN. However, the recent invention of ACE 2, a novel component of the RAAS, Angiotensin peptide metabolism has been given a new direction. Reno-protective fragments (Ang II) may be generated by degrading and dissolving the polypeptide (ACE 2). (1-7). ACE 2 and its fragments' renoprotective action were reduced during DM. Furthermore, persistent hyperglycemia has been shown renal ACE 2 but also its fragments have to be decreased.

Biochemical characteristics

ACE2-dependent mechanisms could also be used to create angiotensin 1-7 straight via angiotensin I. To a large extent it is made in these three places: cardiac and brains, as well as kidney.[2,3] In rats, the plasma half-life of angiotensin 1-7 is only 9

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sec. In the lungs, angiotensin 1-7 is mostly metabolized by ACE. Angiotensin 1-7 may also be degraded into peptide segments by aminopeptidase and NEP.[4]

Angiotensin 1-7 exerts vasodilation, myocardium protecting, antiarrhythmic, antihypertensive and positive inotropic actions in the CVS, as well as suppressing pathogenic heart remodeling.[5] Another notion is that Angiotensin 1-7 reduces insulin resistance, which may improve metabolism. However, whereas most of the impacts appear to be defensive, others seem to be inconsistent. Angiotensin 1-7, for example, could have biphasic impacts on arrhythmias, producing them at large concentrations while avoiding them at low ones.[6] Similarly, some experimental data imply that angiotensin 1-7's biological actions in the kidney are advantageous, while others believe they are harmful.

The bio activities of ANG 1-7 are widely characterized, but the signaling pathways remain unknown. Phosphatidylinositol-3-kinase-Akt is activated by angiotensin 1-7, both in the lab and in the body. Angiotensin 1-7's favorable effects on blood valve function and metabolism may be partly explained by the role of Akt in boosting NO synthase activity as well as insulin signaling. Ang1-7 suppresses activated akt as well as renal proximal tubular cells. Ang II activates MAPKs in these cells, causing oxidative, hypertrophic and proliferative responses; hence, angiotensin 1-7 may counteract those affects. The effects of ANG1-7 on MAPKs, on the other hand, are very cell specific; ANG1-7 enhances both angiotensin-II-induced and basal MAPK activity in certain cell type activation of the Protein Kinase A (PKA)-dependent on cAMP seems to be required for smooth-muscle depolarization generated by ANG1-7-mediated potassium channel opening. Mas is inextricably linked to Gq/11 and the subsequent phospholipase C-protein kinase C signaling pathway. ANG1-7, on the other hand, does not seem to promote Gq signaling in Mas-expressing cells.[7,8]

PKC Pathway

Protein Kinase C beta (PKC) is an endothelial isoform of the threonine/serine kinase family that is involved in the regulation of vascular tone. A slew of data suggested that this enzyme was a major contribution to the endothelial dysfunction reported in diabetic patients. Diacylglycerol (DAG) activates PKC, which is normally obtained as a result of the binding of a ligand to a receptor.[9] It has been postulated that, in the presence of hyperglycemia and high circulating FFA, a unique mechanism for the DAGactivation, including de novo synthesis from glucose, may be used for this purpose.[10] Indiabetes the models of animal, has been discovered that PKC and DAG levels are much greater than normal.[11] When activated, PKC causes a variety of intracellular effects, many of have experimentally found by utilizing ruboxistaurin which is inhibitor of PKC.[12] This includes increased expression of ET-1, VCAM and ICAM, among other proteins.[13] PKC also stimulates the production of NADPH oxidase in the vasculature, which is a substantial Oxygen source and a contributor to oxidative stress in the endothelium. In fact, it has been shown that inhibiting PKC decreases O2 levels in the diabetic vascular.[14] The results of a clinical experiment in healthy individuals revealed that PKC inhibition reduced the impairment of endothelial-dependent vasodilation caused by hyperglycemia.[15] Unfortunately, a subsequent experiment by the same authors revealed that this effect was not seen in individuals with type 2 diabetes, suggesting that further research is needed to clarify the function of PKC in this disease.[16] Additionally impacts of PKC in the endothelium include activation of the Nuclear Factor-B (NFB), changes in the expression of eNOS and the signal pathway of PI3-Kinhibition, among others. Figure 1 shows hypothesis of diabetic nephropathy.

In the modulation of RAAS peripheral activity, the brain acts a vital role. BP, heart failure and renal failure are all caused by RAS in the brain. The increase in sympathetic outflow that occurs when the ACE-Ang II type 1 receptor is overexpressed in the brain has been related to cardiovascular disease. Inhibitors of ACE along with Ang II receptor blockers were proven to be effective in treating brain as well as cardiovascular disorders when administered intracerebroventricularly in animal experimental models, according to many studies. ICV injection of an Enzyme inhibitor or AT1 blocker has also been shown to promote the creation of ACE 2 cerebral as well as Ang (1-7) while reducing central sympathetic outflow as well as peripheral renin and Ang II concentrations in the brain.

PKC activation in diabetic nephropathy

Initial glomerular hyperfiltration, gradual deposition of extracellular matrix in the glomerular mesangium and tubule-interstitium and progressive renal insufficiency are the distinguishing features of diabetic nephropathy. It is believed that metabolic, hemodynamic and probably inflammatory processes caused by hyperglycemia are what are responsible for causing these injuries.

PKC is activated by hyperglycemia, which also leads to an increase in connective tissue growth factor and TGF-b, which in turn causes nephromegaly and mesangium enlargement. The development of glomerular sclerosis and diabetic nephropathy are both outcomes of matrix deposition leading to mesangial enlargement.

Literature review

Investigating melatonin's potential therapeutic usefulness in DN: benefits beyond anti-oxidative stress. Diabetic Nephropathy (DN) is a frequent diabetic consequence and the leading cause of chronic renal failure.[17] The key reasons producing DN damage are physiological/pathological changes caused by excessive glucose, Polyol pathway expansion, accumulation of AGEs and activation of PKC as well as Transforming Growth Factor (TGF) signals

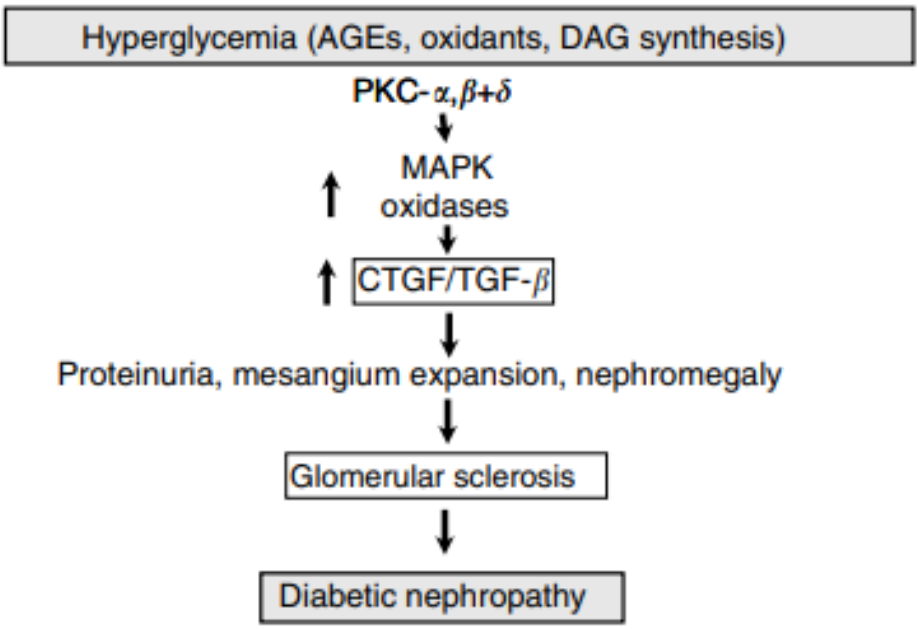

Figure 1: Hypothesis of diabetic nephropathy.

are examples of these changes. The RAS and oxidative stress are also implicated. The pineal gland is the primary source of the sleep-inducing hormone melatonin, which has been related to the development of diabetes. The use of exogenous melatonin has been shown to reduce glucose levels of blood as well as alleviate high glucose-induced disease damage in investigations. Melatonin, on the other hand, has a high antioxidant impact and may suppress RAS activation. As a result, it's important to look into the therapeutic effects and usefulness of melatonin on DN.

In the following study, they studied RAS dysfunction affects TLR4 activity in the kidney, which helps us understand the DN etiology. Insights into the interaction among RAS and TLR4 that promotes the improvement of DN that results to the development of novel therapeutics. In order to fully understand how DN works, further research is required. Innate immunity and the Renin-Angiotensin System (RAS) both contribute significantly to the pathogenesis of DN.[18] Apart from its conventional roles, improperly regulated RAS has been implicated in the inflammatory process associated with DN. TLR4 is the innate immune system's best studied pattern recognition receptor and also its activation has been linked to the development of DN.

Aims and objective

The goal of this investigation is to find out if chronic hyperglycemia-induced nephropathy in wistar rats is caused by cerebral angiotensin (1-7) levels.

The renin-angiotensin system's Ang (1-7) component has been discovered to have physiological activity (RAS). Globally, Diabetic Nephropathy (DN) bridges for ESRD, decreases Ang (1-7) peripheral activity. The brain's RAS regulates the activity of RAS throughout the body's peripheral nervous system.

MATERIALS AND METHODS

Animals

In these experiments, Wistar rats of any gender with mass of 180-250 g were employed. In compliance with the national standard utilized in the laboratory animals, The Laboratory Animal Ethics Committee approved the experimental approach used in this study (IAEC/NIET/2022/02/37). All the rats were kept in one place on a regular light/dark cycle and had unlimited access to a normal chow meal and H_2O. In metabolic cages, the animals were kept. Each rat's urine was taken over a 24 hr to conduct laboratory research.

Chemicals and drugs

Angiotensin (1-7) and Aliskerin were created in artificial cerebrospinal fluid and administered for 2 weeks through Intracerebroventricular (ICV) administration, 10 weeks following streptozotocin injection. All of the remaining chemicals utilized in this investigation were analytical grade and were produced fresh each time they were used.

Surgery with intracerebroventricular aliskerin and angiotensin-converting enzyme inhibitors (1-7).

The animal was positioned in a stereotaxic apparatus after being sedated with thiopental sodium (30 mg/kg). After the head was put on a frame, a midline sagittal cut is made in the scalp. Using a Hamilton micro-syringe, a polypropylene cannula was implanted into each of the two lateral cerebral ventricles via two perforations in the skull. According to the coordinates, the ICV

cannula was inserted 0.8 mM posterior to the lamda, 1.8 mM lateral to a sagittal suture and 3.6 mM. After all the procedure the cut covered with stiches. During the postoperative time, special attention was shown to the animals, with food and drink provided inside the rats' cage. A synthetic cerebrospinal fluid comprising 1.47 NaCl, 2.9% K+, 1.66 Mg+2, 1.73 Ca+2, as well as 2.2 dextrose was administered to rats for two weeks. Aliskerin (100 nmol/day, upto 2 weeks) and (1-7) (4.8 mol/day, upto 2 weeks) were both administered through intravenous injection and had identical outcomes. Using a Hamilton micro-syringe were delivered in a volume of 5l (ACSF) in both lateral ventricles alone or in combination. 2 min after insertion of the Hamilton micro-syringe. The cannula was kept in place for the administered the drug.

Induction and evaluation of disease

Experimental hyperglycemia was employed a single dose of Streptozotocin (STZ) (35 mg/kg, i.p.) to create an experimental model of Diabetes Mellitus (DM). Hyperglycaemia was defined as a blood serum glucose level of >200 mg/dL in diabetic rats. The Glucose Oxidase-Peroxidase (GOD-POD) approach was used to assess blood glucose levels collected from the retro orbital sinus using a commercial kit.

Disease parameter evaluation

Conventional diagnostic kits were used to detect the levels of blood protein in the urine, serum creatinine, as well as protein in urine to identify diabetes mellitus-induced nephropathy.

The amount of nitrite in the blood is calculated

Unlike NO, nitrite is readily measured, and the concentration of nitrite may be used to estimate the quantity of NO generation. The amount of nitrite released in coronary effluent was determined. 0.5 mL Greiss reagent was added to 0.5 mL serum. Using a spectrophotometer, the optical density at 550 nm was determined. By comparing the reading from the spectrophotometer of the standard solution, the nitrite concentration was determined. The outcome was measured in µmoles/L.

A weight-to-kidney ratio was obtained by dissecting the kidneys and removing their fasciae as well as weighing each kidney separately. The following method was used to compute kidney weight/body weight (percentage).

$$kidney\ weight / body\ weight = \frac{left\ kidney\ weight - right\ kidney\ weight}{body\ weight} \times 100$$

Truce for the experiment

36 rats were divided into the following experimental groups, each with six individuals

Set I: Negative Control, the rats were allowed to eat and drink normally.

Set II: Positive Control, animals were given 35 mg/kg i.p. of streptozotocin to induce diabetes mellitus.

ACF 5l in each hemisphere was administered to animals

Set III: Vehicle control- for two weeks after 7 days of streptozotocin administration'.

Set IV: ICV treatment diabetes group mice received 4.8 g/day of Angiotensin (1-7) by ICV after seven days of streptozotocin administration.

Set V: At a dose of 100 nmol/day for two weeks, mice in set after 7 days of streptozotocin administration.

Set VI: ICV were given aliskerin (100 nmol/day) and Ang (1-7) (4.8 g/day) in combination via ICV for 2 weeks after receiving streptozotocin".

Analytical statistics

All the data is represented by the mean and standard deviation. An ANOVA and Tukey's multiple comparisons test were used to examine the data from separate groups. A statistically significant $p<0.05$ was used.

Results

At the commencement of the experimental study, numerous parameters were evaluated. Up to the tenth week following streptozotocin administration, all parameters were examined and the findings were made comparison among the ground which takes the treatment and normal and diabetic control groups. At 2 weeks, diabetic nephropathy was diagnosed and therapy was maintained until the 10th week.

Effects of pharmaceutical treatments on glucose levels in the blood

Animals were given one dose of STZ, the serum blood glucose levels increased considerably ($p<0.05$) when compares to normal rats. After ten weeks of streptozotocin insertion, during experimental work after 4 weeks Ang (1-7) (4.8 g/day) as well as Aliskerin (100 nmol/day) given in combination treatment, would have no impact on diabetes animals' blood sugar levels (Figure 2A). 20 µL of serum to 1500 µL of working glucose reagent and incubation at 37°C for 10 min, 1500 µL of filtered water were added to the test samples that were ready. To prepare the standard sample, add 20 µL of reagent 3 to 1500 µL of working glucose reagent and 1500 µL of purified water. To prepare the blank, 1500 µL of working glucose reagent was mixed with 1500 µL of filtered water. The reagents were combined and after 10 min of room temperature incubation, the absorbance of the test and standard was measured spectrophotometrically at 505 nm against a blank. Using a formula, the serum glucose level was determined.

All values were reported as mean±Standard Deviation (SD). The statistical analysis was carried by utilising Graphpad and Sigmastat software. One way Analysis of Variance (ANOVA) was used to evaluate the data from the different groups. $p<0.05$ was deemed to be substantially static. Calibration curve shows the concentration of drugs in serum (Figure 3).

All the parameters are recorded and are all affected by pharmaceutical treatments

Blood sample were withdrawn from the retro orbital sinus of rat. The increased blood urea nitrogen, protein urine and urinary output volume were seen in rats given streptozotocin (35 mg/kg) ($p<0.05$). For two weeks after eight weeks of streptozotocin administration, therapy with Ang (1-7) and aliskerin alone or in combination significantly ($p<0.05$) decreases elevated BUN, protein in urine, overall urinary output volume in diabetic rats. To illustrate, have a look at Figures 2B, 2C and 2D.

Pharmacological treatments have an effect on serum nitrite levels

Nitrite concentrations in serum were found to be considerably ($p<0.05$) lower in diabetic rats than in normal rats. After 10 weeks of streptozotocin administration, when treated with drugs as mentioned substantially ($p<0.05$) enhanced the quantity of nitrite in the serum of diabetic rats (Figure 2E).

Pharmacological therapies influence kidney mass and the kidney mass/body mass ratio.

When diabetic animals were made in comparison to normal ones, a substantial rise in the kidney mass/body mass ratio was observed. For two weeks after streptozotocin administration and when treated with drugs (Ang 1-7) and aliskerin in alone and in combination results shown substantially increased kidney mass/body mass ratio when compares to diabetic rats (Figure 2F).

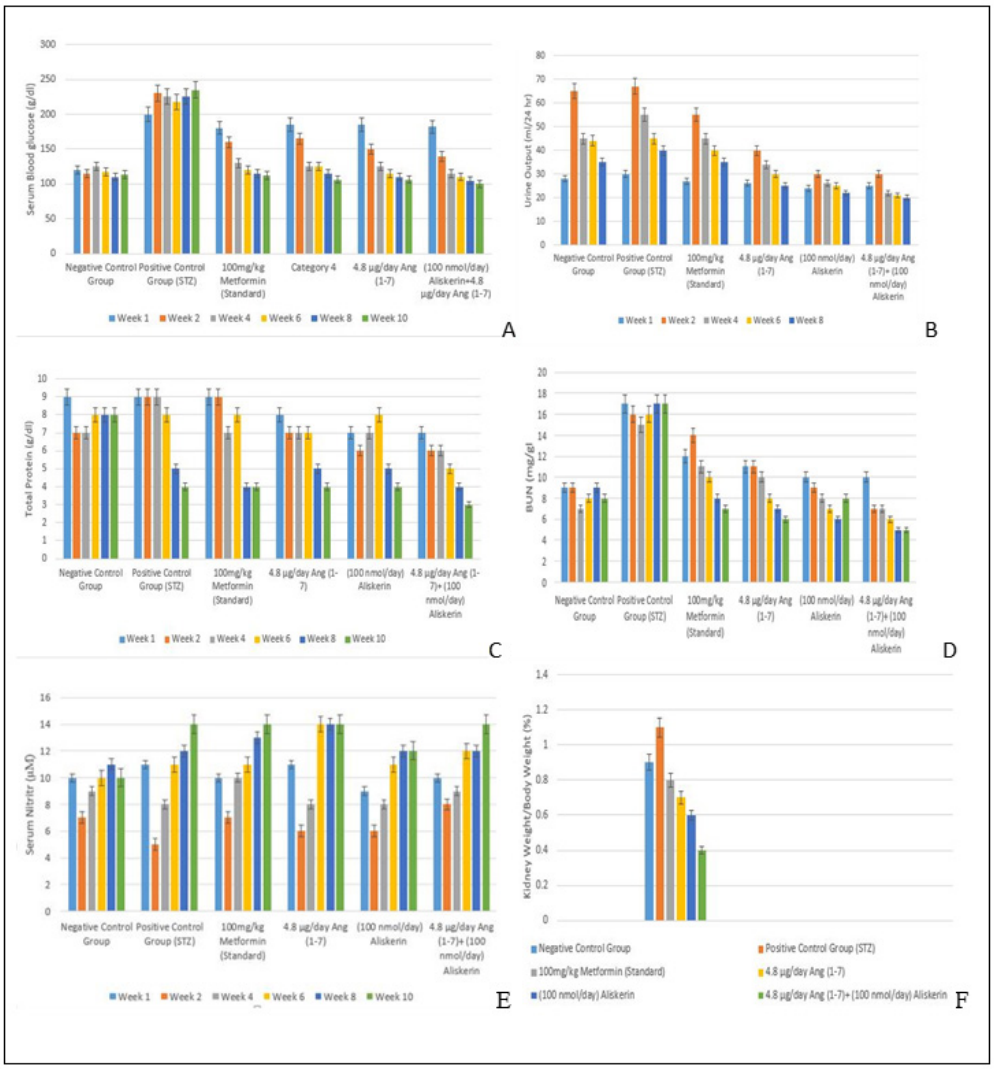

Figure 2: (A) Blood Glucose Level (B) Urine Output (C) Total Protein (D) Blood Urea Nitrogen (E) Serum Nitrite (F) Kidney Weight.

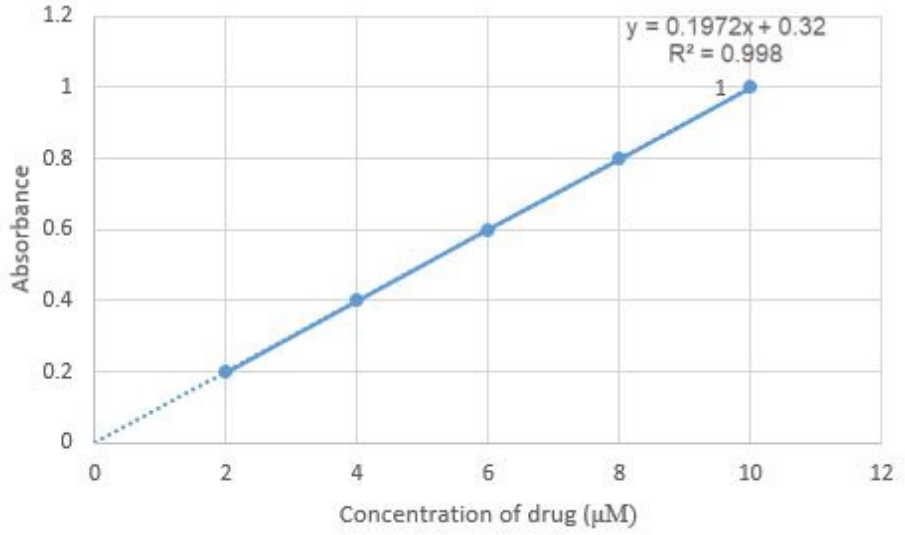

Figure 3: Calibration Curve of drug.

RESULTS

Aliskerin, a direct renin inhibitor, significantly reduces blood pressure and inhibits sympathetic overactivity when administered centrally.[19-22] The vasoconstriction enzyme and its brain traces play an essential function in regulating the activity of the RAAS in the peripheral nervous system.[23] A decrease in angiotensin-II peripheral activity and a reduction in sympathetic outflow have been seen with brain overexpression of ACE 2 and Ang (1-7).[24,25] Renal damage caused by Ang II is thought to be inhibited by Ang (1-7).[26] Renin-angiotensin system is disrupted in DN by elevated levels of RACE and Angiotensin II, therefore in diabetes, the levels of this enzyme are lowered.[27] But it has been shown that blocking the AT1R in the brain by administering ACE inhibitors or AT1 blockers Intravenously (IV) increases the production of cerebral ACE 2 and Ang (1-7) and decreases the sympathetic outflow and plasma levels of rennin and Ang II in the central nervous system. Thirty-two and a half as evidenced by lower levels of serum creatinine, kidney-to-body weight ratio and all other parameters decreased and the level and elevates the serum nitrite in our study; two weeks of ICV administration of Ang (1-7) inhibited the progression of the DN. When it came to improving renal function, aliskerin (an AT1R blocker) was shown to be more effective than Ang (1-7). Therapy with aliskerin and Ang (1-7) in combination was more effective than treatment with either medication alone. The reduced central and peripheral actions of Ang II may be responsible for the observed Reno protective effects. In diabetic rats, treatment with aliskerin and Ang (1-7) did not have any impact on the raised level of blood glucose, which suggests that the Reno protective action of both drugs may be independent. The experimental design of this investigation clearly shown that administration of both the drugs in single or in combination shows positives results to treat diabetic nephropathy by inactive or lowers the peripheral activity of the RAAS, which was clearly represents by various biochemical parameters performed during this study.

DISCUSSION

When given a single dosage of streptozotocin (120 mg/kg ip), blood glucose levels rise significantly. Serum creatinine, blood urea nitrogen and proteinuria are all known nephropathy indicators.[28] A significant rise in the serum creatinine, blood urea and urine protein concentration was seen after the administration of streptozotocin.[29,30] It has been shown that DN may be diagnosed by a drop-in serum nitrite concentration and an increase in the kidney weight/body weight ratio.[31] An increase in the synthesis of renal endothelin-1 (eNOS) has been seen in patients with longstanding diabetes mellitus.[32] In this research, diabetic rats had a higher kidney-to-bodyweight ratio than normal rats, although their serum nitrite content was lower. DN causes an over-activation of the peripheral RAAS.[33] After administration of Ang (1-7) and aliskerin in alone and in combination shows the decrement of serum blood glucose and other parameters. The reason behind the results are angiotensin converting enzyme (ACE) produces angiotensin II (Ang II), a powerful vasoconstrictor, as the primary impact of RAAS. Increased Ang-II levels in the bloodstream further worsen the release of renin from the kidney by lowering its blood supply and glomerular damage, which leads to nephropathy. ACE 2 is one of the novel elements of the RAS.[34,35] Reduced the threshold of constriction enzymes and its fragments in the kidney promote renin release.[36] Aliskerin which is direct renin inhibitor with Ang (1-7) was shown to be reduced in diabetes mellitus. Furthermore, persistent hyperglycemia has been shown to reduce renal ACE 2 and its fragments concentrations.[37,38]

There is also a local autonomous RAS in the brain, in addition to the system in the periphery.[39] However; we found that in our

work, we discovered that the peripheral renin angiotensin system is regulated by brain systems. According to studies from other labs, the brain renin angiotensin system is linked to hypertension, renal failure and heart failure. This is corroborated by the findings.

CONCLUSION

Ang (1-7) and aliskerin, which don't really pass the BBB, were found to lessen the rise in diabetic nephropathy-induced all the levels and all variables that may be used to measure renal function over time, increased serum nitrite following ICV administration. These Reno protective effects might be a result of Ang II's reduced central sympathetic outflow and peripheral activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ACE: Angiotensin Converting Enzyme; **BUN:** Blood Urea Nitrogen; **BBB:** Blood Brain Barrier; **DN:** Diabetic Nephropathy; **ESRD:** End Stage Renal Disease: **GFR:** Glomerular Filtration Rate; **ICV:** Intracerebral ventricle; **ip:** Intraperitoneal; **MAPK:** Mitogen Activated Protein Kinase; **PKA:** Protein Kinase A; **PKC:** Protein Kinase C; **RACE:** Renin Angiotensin Converting Enzyme; **RAS:** Renin Angiotensin System; **MAPK:** Mitogen Activated Protein Kinase.

SUMMARY

One of the world's most debilitating diseases, Diabetes Mellitus (DM) affects millions of individuals. Insulin resistance or insulin insufficiency is the hallmarks of Diabetes Mellitus (DM), which results in increase in glucose concentration and changes in glucose, lipid and metabolism of protein.

A sustained rise in albumin levels in the urine, an increase in the Glomerular Filtration Rate (GFR), Glial hypotrophy, tubule interstitial fibromatosis, mesangial hypertrophy as well as podocyte loss are all signs of DN in addition to increased extracellular matrix protein deposition.

The recent invention of ACE 2, a novel component of the RAAS, Angiotensin peptide metabolism has been given a new direction. Reno-protective fragments (Ang II) may be generated by degrading and dissolving the polypeptide (ACE 2). (1-7). ACE 2 and its fragments' renoprotective action were reduced during DM. Furthermore, persistent hyperglycemia has been shown renal ACE 2 but also its fragments have to be decreased.

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