BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902023e20200

Article

# Role of Brain Angiotensin-II in Development of Experimental Diabetic Nephropathy in Wistar Rats

**Anubhav Kumar Singh[1]*, Niraj Kumar Singh[1], Ahsas Goyal[1], Bhupesh Chander Semwal[1], Harlokesh Narayan Yadav[2]**

*[1]Institute of Pharmaceutical Research, GLA University 17-Km Stone NH-2, Mathura-Delhi Highway, Chaumuhan, Uttar Pradesh, India, [2]All India Institute of Medical Sciences (AIIMS), Gautam Nagar, Ansari Nagar East, New Delhi, India*

The renin-angiotensin-aldosterone system (RAAS) plays a key role in diabetic nephropathy (DN). Angiotensin-II secreted during the RAAS pathway increases nephropathy. It stimulates oxidative stress which can quench nitric oxide. Reduced nitric oxide level aggravates Ang-II-induced vasoconstriction. Ang-II has also emerged as a central mediator of the glomerular hemodynamic changes that are associated with renal injury. Deletion of ACE2 is also noted due to increased Ang-II level which leads to the development of DN. We hypothesize that nephropathy caused by Ang-II in the periphery may be controlled by brain RAAS. ACE inhibitors and ARBs may show the renoprotective effect when administered through ICV without crossing the blood-brain barrier. DN was observed after 8 weeks of diabetes induction through alloxan. Administration of captopril and valsartan once and in combined therapy for 2 weeks, significantly reduced urine output, blood urea nitrogen, total protein in the urine, serum cholesterol, serum creatinine, serum triglycerides, and kidney/body weight ratio as compared to diabetic control rats. Further, combination therapy significantly increased the body weight and serum nitrate level as compared to diabetic control animals. However, increased ACE2 levels in the brain may reduce the sympathetic outflow and might have decreased the peripheral activity of Ang-II which shows beneficial effects in DN.

**Keywords:** Diabetic nephropathy. Brain RAAS. Intracerebroventricular injection. Angiotensin Converting Enzyme.

## INTRODUCTION

Recent studies say that globally around 382 million people are suffering from diabetes which will rise to 592 million by 2035 and will become the most challenging health-related problem of the 21st century (Bertoncello *et al.*, 2015). India is also known as the diabetic capital of the world as it projected 109 million diabetic individuals by 2035 (Kaveeshwar, Cornwall, 2014). Currently, diabetic mellitus (DM) affects more than 62 million people in India which is more than 7.1% of the total population. The estimated survey shows that around 1 million people die in India due to diabetic complications every year (Kaveeshwar, Cornwall, 2014; Unnikrishnan *et al.,* 2007). Diabetic nephropathy (DN) remains the most common cause of end-stage renal disease (ESRD) worldwide (Zhang *et al*., 2015). Patients suffering from DN have symptoms like proteinuria & microalbuminuria and show a progressive decline in glomerular function. DN is a serious chronic complication in type 1 diabetes mellitus. Along with kidney complications nephropathy caused by diabetes also causes a rise in arterial blood pressure which gets develops in 30 to 40 % of all types of diabetic patients (Lewis *et al*., 1993; Trevisan, Dodesini, Lepore, 2009).

The exact mechanism of DN is not very much clear but it has been well reported that the renin-angiotensin-aldosterone system (RAAS) plays a major role in the regulation of DN and that is why the first line treatment therapy includes blockade of the RAAS pathway to treat

***Correspondence:** A. K. Singh. Division of Pharmacology. Institute of Pharmaceutical Research. GLA University. 17-Km Stone NH-2, Mathura-Delhi Highway, Chaumuhan. Mathura, 281406, Uttar Pradesh, India. Phone: +918958452030. Email: anubhavs01@gmail.com. ORCID: https//orcid.org/0000-0002-6687-3686

DN (Trojacanec *et al*., 2013). Angiotensin-II (Ang-II) one of the main components of RAAS shows an important role in both renal physiology and the pathogenesis of chronic kidney disease (Kobori *et al.,* 2013). The Ang-II of the RAAS gets increased in DN. Ang-II affects the kidney in many ways such as it causes cellular differentiation, cell proliferation, apoptosis, renal hypertrophy, tubular sodium restoration, and most importantly vasoconstriction (Leehey *et al*., 2000).

The RAAS pathway is present in various organs, including the heart, adipose tissues, kidney, adrenal gland, and brain. It has been well documented that brain RAAS plays a critical role in the development of kidney failure and heart failure (Mckinley *et al*., 2001; Chawla, Sharma, Singh, 2010). Angiotensinogen is common the precursor molecule for the synthesis of angiotensin I, II, and III in the brain as well as in the liver (Campbell *et al*., 2003). The RAAS pathway starts from the release of renin from the kidney after which this renin acts on plasma angiotensinogen to convert it into angiotensin-I (Ang-I). Angiotensin-converting enzyme (ACE) converts Ang-I into Ang-II which shows a deleterious effect on the kidney to cause renal damage (Doughue *et al*., 2000; Crackower *et al*., 2002). After that formation of angiotensin-converting enzyme-2 (ACE-2) takes place from ACE, this is a monocarboxypeptidase compound. This ACE-2 is one of the new elements of the RAAS that cleaves decapeptide Ang-I and octapeptide Ang-II to form Angiotensin (1-7) & Angiotensin (1-9) respectively (Tikellis *et al*., 2008). When ACE2 removes amino acid from the carboxy-terminal of Ang-II, Ang (1-7) gets synthesized. Ang (1-7) which is a heptapeptide compound shows beneficial effect in DN as it has opposite actions than Ang-II (Battle, Soler, Ye, 2010).

Renin and Ang-II secreted during the RAAS pathway increase nephropathy as it shows rapid effects like increased vasoconstriction and increased aldosterone secretion (Santos *et al*., 2013; Sharma, Sharma, 2013). Ang-II stimulates oxidative stress which can quench nitric oxide, an endothelium-dependent vascular relaxant, and thereby aggravate Ang-II-induced vasoconstriction (Ruster, Wolf, 2006). Ang-II also emerged as a central mediator of the glomerular hemodynamic changes associated with progressive renal injury (Alberti, Zimmet, Shaw, 2006). Ang (1-7) and Ang (1-9) counter-regulate the negative consequences of Ang-II by the activation of Mas receptors (Giani, Munoz, Pons, 2011). Moreover, the deletion of ACE2 is noted due to increasing Ang-II levels which lead to the development of DN (Zini *et al*., 1996).

The ACE2 enzyme found in the brain plays an important role in the regulation of peripheral activity of RAAS (Baltatu, Campos, Bader, 2011). It has been believed that over-expression of ACE2 in the brain reduces the sympathetic outflow and decreases the peripheral activity of Ang-II (Feng *et al*., 2010).

However, in this study, we want to investigate how brain RAAS may control the periphery RAAS pathway without crossing the blood-brain barrier itself. Hence, in this research work, we have hypothesized that nephropathy caused by angiotensin-II in the periphery may be controlled by brain RAAS. Drugs like ACE inhibitors and ARBs may show a renoprotective effect when administered through ICV-route.

## MATERIAL AND METHODS

### Animals

A total 36 Wistar rats of either sex with the body weight of 200g ± 10% were used in this study. Animals were randomly divided into six groups (Normal Control, Diabetic Control, Vehicle treated, Captopril treated, Valsartan Treated, and Captopril + Valsartan Treated) having six rats in each group. Rats were fed with standard food and water *ad libitum*. The animals were housed in cages with a 12 h light/dark cycle and temperature-controlled environment (20±2$^{O}$C). The experimental protocol was approved by *Institutional Animal Ethical Committee* (GLAIPR/CPCSEA/IAEC/2015/P.Col/R1) for the use of laboratory animals.

### Drugs and Chemicals

Alloxan monohydrate, Valsartan, and Captopril (Sigma-Aldrich, Hyderabad, India) were freshly prepared before the administration. All the other regents used in the present study were analytical grade and freshly prepared before use.

### Surgery and administration of drugs

Thiopental sodium (Thiosol Sodium, Neon laboratories ltd, Mumbai; 30 mg/Kg, *i.p*) was used to anesthetize the rats (Kushwaha *et al.,* 2011). A minor cut (approx 1-2cm) was applied on the head on either side of the midline (Haley, McCormick, 1957). Extra tissues were removed to expose the skull. After that rats were placed on stereotaxic apparatus to determine the ICV position. The location concerning the lambda point was 0.8mm Anteroposterior (AP), 1.8mm Mediolateral (ML), and 3.6mm Dorso-ventral (DV) deep to the skull surface. After the ICV point was determined, the drilling was done manually by using a driller in both hemispheres, and polypropylene cannula were placed in each hole for smooth administration of drugs, regularly for 2 weeks. The drug was administered by using a Hamilton syringe at 1µL/min rate and 5µL drug or vehicle was introduced in each hemisphere.

Vehicle treated group received artificial cerebrospinal fluid (aCSF); NaCl- 8.66g, KCl- 0.224g, CaCl2.2H2O- 0.206g, MgCl2.6H2O- 0.163g in 500ml of distilled water + NaH2PO4.7H2O- 0.214g, NaH2PO4. H2O- 0.027g in 500ml distilled water to make 1L of solution) in a volume of 5µL in the lateral ventricles by ICV route in both hemispheres through the cannula (Zini *et al.,* 1996). Similarly, Captopril (2µg/day ICV; Baum, Becker, Suberts, 1983) and Valsartan (100 nmol/day ICV; Nakamura *et al.,* 2014) in single dosage form and combined form were given in a volume of 5µL in both lateral ventricles for two weeks daily.

### Inductions and assessment of experimental diabetic nephropathy

Randomly selected rats were allocated to receive alloxan monohydrate (120 mg/Kg, *i.p.*; Once) for induction of experimental type 1 diabetes mellitus (Sharma *et al*., 2010). Animals having serum glucose levels more than 200 mg/dL were considered as diabetic. Blood samples were collected from the retro-orbital sinus. The presence of diabetes mellitus was confirmed by measurement of serum glucose level by GOD-POD method (Trinder, 1969) using a commercially available enzymatic kit (Span diagnostics ltd., Surat, India).

### Assessment of diabetic nephropathy

After 8 weeks of diabetes induction, DN was confirmed. It was evaluated biochemically by accessing the blood urea nitrogen (BUN), serum creatinine, total protein in the urine, and serum nitrite level weight (Vaishya, Singh, Lal, 2008) using diagnostic kits (Span diagnostics ltd., Surat, India).

#### *Estimation of serum nitrates*

Unlike NO, nitrite can be measured easily and nitrite concentration can be used to infer the level of NO production. Nitrite release in coronary effluent was measured. Greiss reagent 0.5 ml (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride in water) was added to 0.5 ml of serum. The optical density at 550 nm was measured using a spectrophotometer. Nitrite concentration was calculated by comparison with the spectrophotometer reading of standard sodium nitrite. The result was expressed as micromoles per liter (µM/L).

#### *Estimation of serum cholesterol and triglyceride*

The serum cholesterol and triglyceride were estimated by blood samples collected by retro-orbital sinus by using commercially available kits (Span Diagnostics Ltd., Surat, India).

#### *Estimation of kidney weight/body weight ratio*

Kidney weight/Body weight ratio was estimated by removal of both kidneys, renal fascias were removed and kidneys were weighed individually. The kidney weight/body weight (%) was calculated by following the formula (Sinuani *et al*., 2006).

Kidney weight/Body weight = (Left kidney weight – Right kidney weight)*100/Body weight

### Statistical analysis

All values were expressed as mean ± standard deviation (S.D.). Statistical analysis was performed by using GraphPad and Sigmastat software. Data obtained from various groups were significantly analyzed using one-way ANOVA, $p<0.05$ was considered to be statistically significant.

## RESULTS

At the start of the experiment, an assessment of different parameters was done. After a single administration of alloxan (120mg/Kg, *i.p.*), parameters were accessed each week till the 10th week. DN was confirmed at the 8th week and treatments were continued further up to the 10th week after alloxan administration. All parameters were accessed up to the 10th week and findings were compared between the treatment group vs. normal and diabetic control group.

### Effect of various pharmacological interventions on body weight

The effect of treatments on body weight was illustrated in Figure 1 (A). Significant ($p<0.05$) change was found in the body weight of diabetic rats after the administration of alloxan monohydrate as compared to normal rats. Vehicle-treated group rats do not show any significant decrease in body weight when compared to diabetic control rats. Drug-treated groups i.e. captopril ICV treated group (2µg/day) & valsartan ICV treated group (100 nmol/day) alone and in combination shows significant ($p<0.05$) increase in body weight when they are compared to the diabetic control group after treatment.

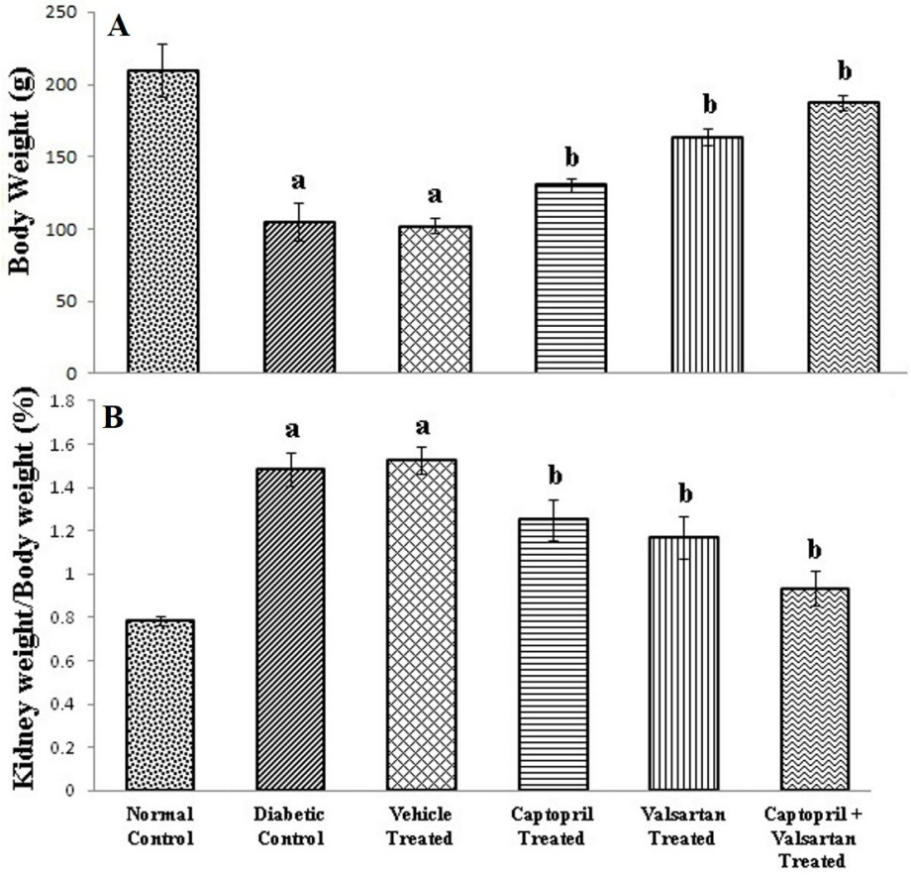


**FIGURE 1 -** Effect of vehicle and drug treatment on body weight (A) and kidney weight/body weight % (B) on 10th week. Values are expressed as mean ± S.D., a=$p<0.05$ *vs*. normal control; b=$p<0.05$ *vs*. diabetic control.

### Effect of various pharmacological interventions on kidney weight/body weight

Figure 1 (B) depicted the effect of treatment on the kidney weight/body weight ratio. A significant increase in kidney weight /body weight was noted in diabetic rats as compared to normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p$<0.05) reduced the elevated kidney weight/body weight ratio as compared to diabetic rats.

### Effect of various pharmacological interventions on blood urea nitrogen

The effect of treatments on BUN was illustrated in Figure 2 (A). The concentration of BUN significantly ($p$<0.05) increases in diabetic rats as compared with normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p$<0.05) reduces the elevated level of BUN as compared to diabetic rats.

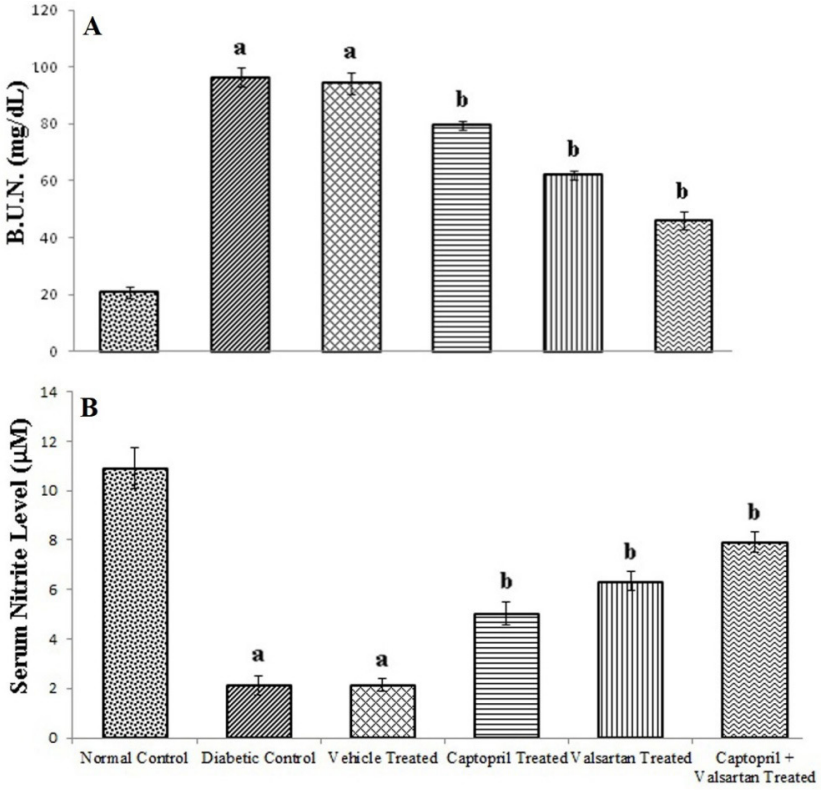


**FIGURE 2 -** Effect of vehicle and drug treatment on blood urea nitrogen (BUN; A) and serum nitrite (B) level on 10th Week. Values are expressed as mean ± S.D., a=$p$<0.05 *vs*. normal control; b=$p$<0.05 *vs*. diabetic control.

### Effect of various pharmacological interventions on serum nitrite level

Figure 2 (B) depicted the effect of treatment on serum nitrite levels. The serum nitrite concentration reduces significantly ($p$<0.05) in diabetic rats when compared to normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p$<0.05) elevates the low serum nitrite level as compared to diabetic rats.

### Effect of various pharmacological interventions on serum total cholesterol

The effect of treatments on serum cholesterol levels was illustrated in Figure 3 (A). A significant ($p$<0.05) increase in serum cholesterol was noted in diabetic rats when compared with normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p$<0.05) reduces the elevated level of serum cholesterol as compared to diabetic rats.

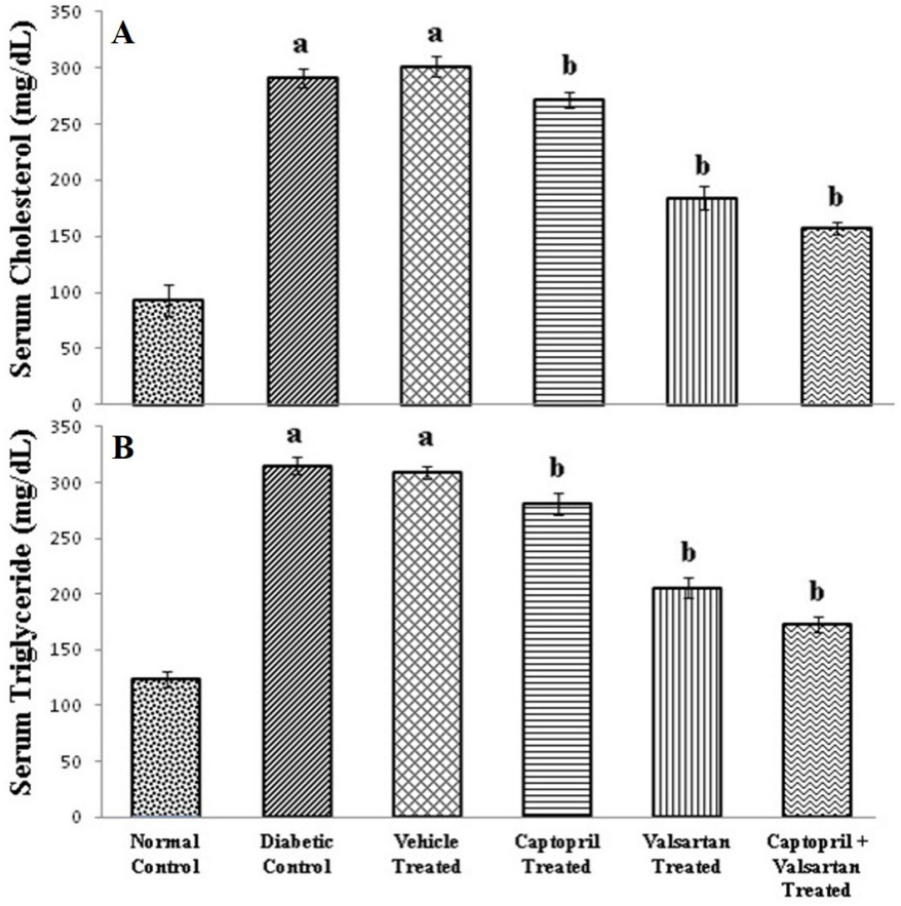


**FIGURE 3 -** Effect of vehicle and drug treatment on serum cholesterol (A) and triglyceride (B) level on 10th Week. Values are expressed as mean ± S.D., a=$p$<0.05 *vs*. normal control; b=$p$<0.05 *vs*. diabetic control.

### Effect of various pharmacological interventions on serum triglyceride level

Figure 3 (B) depicted the effect of treatment on serum triglyceride levels. Serum triglyceride levels also get elevated significantly ($p$<0.05) in diabetic rats when compared with normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p$<0.05) reduces the high serum triglyceride level as compared to diabetic rats.

### Effect of various pharmacological interventions on urine output/24-hour

The effect of treatments on serum urine output/24-hour was illustrated in Figure 4 (A). Administration of

alloxan (120 mg/Kg; *i.p.*) significantly ($p<0.05$) increases the urine output as compared to normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p<0.05$) reduces the elevated urine output in diabetic rats.

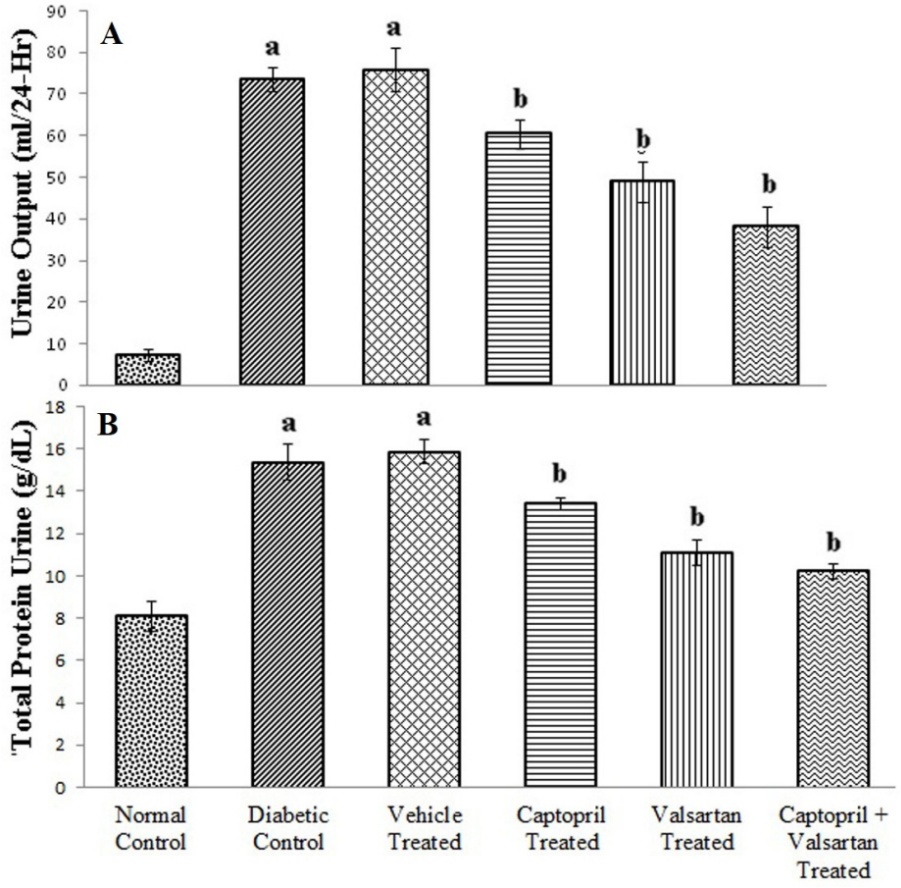


**FIGURE 4 -** Effect of vehicle and drug treatment on urine output/24-hr (A) and total protein in the urine (B) on 10th Week. Values are expressed as mean ± S.D., a=$p<0.05$ *vs*. normal control; b=$p<0.05$ *vs*. diabetic control.

### Effect of various pharmacological interventions on total protein in urine

Figure 4 (B) depicted the effect of treatment on total protein in the urine. The concentration of total protein in urine significantly ($p<0.05$) increases in diabetic rats as compared with normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p<0.05$) reduces the elevated level of total protein in the urine as compared to diabetic rats.

### Effect of various pharmacological interventions on serum glucose level

The effect of treatments on serum glucose levels was illustrated in Figure 5 (A). A single administration of alloxan (120 mg/Kg; *i.p.*) significantly ($p<0.05$) increases the serum glucose concentration as compared with normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration does not affect the serum glucose level in diabetic rats.

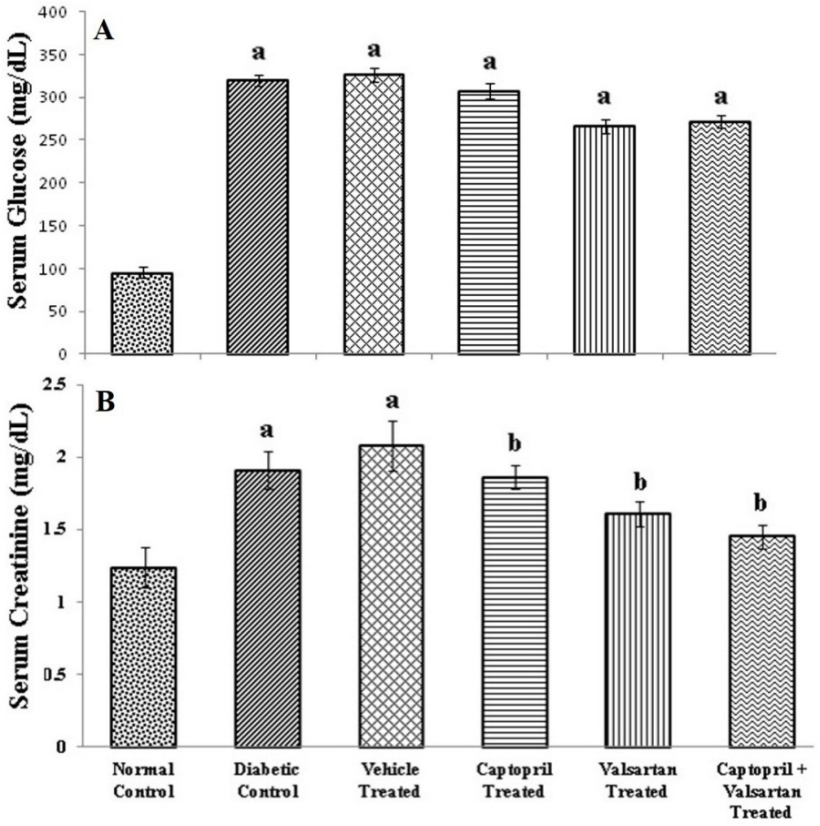


**FIGURE 5 -** Effect of vehicle and drug treatment on serum glucose (A) and creatinine (B) level on 10th Week. Values are expressed as mean ± S.D., a=$p$<0.05 *vs*. normal control.

### Effect of various pharmacological interventions on serum creatinine

Figure 5 (B) depicted the effect of treatment on serum creatinine. Serum creatinine concentration was significantly ($p$<0.05) increased in diabetic rats as compared with normal rats. Treatment with captopril (2µg/day; ICV) and valsartan (100 nmol/day; ICV) alone or in combination for 2 weeks after 8 weeks of alloxan administration significantly ($p$<0.05) reduces the elevated serum creatinine level as compared to diabetic rats.

## DISCUSSION

In this study, it has been demonstrated for the first time that brain RAAS may control the DN. Diabetes mellitus has been induced by a single administration of alloxan at 120 mg/Kg *i.p.* (Sharma *et al.*, 2010) which develops nephropathy in 8 weeks. Assessment of parameters was continued up to the 10th week. In the present study, ACE Inhibitor captopril at the dose of 2 µg/day; ICV (Baum, Becker, Suberts, 1983) and ARB valsartan at the dose of 100nmol/day; ICV (Nakamura *et al.,* 2014) were administered to rats. Captopril and valsartan administered through the ICV route may control the peripheral activity of Ang-II by reducing the sympathetic outflow without crossing the blood-brain barrier (Feng *et al.*, 2010). Rats of the vehicle-treated group receive ACF through the ICV route which does not show any significant change as compared to the diabetic control group. While captopril and valsartan either alone or in combined therapy show subsequent renoprotection in severe nephropathic rats.

Alloxan is a kind of β-cytotoxin that damages β-cells of the pancreatic islet which leads to insufficient insulin release that causes type-1 diabetes. Alloxan generates ROS which preferentially accumulates in β-cells through the GLUT2 glucose transporter to destroy it. Treatment with

captopril and valsartan alone or in combined form does not show any significant change in increased glucose level which has been supported by Onozato *et al*., 2002. In type 1 diabetes mild reduction in body weight is common due to flushing of glucose & calories through urine (Rosenfalck *et al*., 2002). Treatment with captopril, valsartan, and their combination shows significant alteration in body weight as compared to the diabetic control group.

DN causes many characteristic changes to the structure of the glomerulus mainly known as glomerulosclerosis (Cockcroft, Gault, 1976). Due to nephropathy, extracellular deposits get increased inside the renal corpuscle, with decreased surface area for filtration. It also makes the glomerular basement membrane thicker and leakier, due to which polyuria occurs (Makaya *et al*., 2013). The diabetic control group significantly increases the urine output as compared to the normal group. While captopril & valsartan alone or in combined therapy significantly reduces the elevated urine output. Restoration in glomerular basement membrane permeability may be the reason for it.

The serum creatinine, BUN, and urinary total protein level are commonly used parameters to check the kidney function in DN which gets abnormally elevated in the case of DN. Serum creatinine is the most important indicator that shows renal health because it is a byproduct of muscle metabolism that is excreted by kidneys (Bakris, Weir, 2000; Maschio *et al*., 1996). A damaged kidney was unable to filter creatinine which causes increased serum creatinine levels (Zatz *et al*., 1987). Urea is produced by the liver in the urea cycle as a waste product of digested protein (Viberti *et al*., 1994).In nephropathic conditions, the kidney fails to remove excess urea from the body which causes elevation of BUN. Macroalbuminuria is the most common cause of DN. Alteration in kidney function causes excess albumin secretion in urine which is commonly known as proteinuria (Kelly *et al*., 2007; Mogenson, 1987). It has been seen that persistent albuminuria causes dysfunction of several cells like glomerular endothelial cells, podocytes, and proximal tubule epithelial cells due to which proteinuria occurs in DN (Fioretto, Stechower, Mauer, 1998). BUN, serum creatinine &urinary protein level were significantly reduced by alone or combined treatment with captopril & valsartan through the ICV route. This may be due to improved glomerular filtration rate & reduction in glomerular permeability due to which protein loss gets reduced (Chawla, Sharma, Singh, 2010).

Assessment of lipid profile has been done to determine the effect of nephropathy on serum cholesterol level and serum triglyceride level. It was observed that diabetic rat shows a marked increase in both cholesterol and triglyceride levels as compared to normal rats. Dyslipidemia due to diabetes tends to cause hypercholesterolemia in which LDL, VLDL, and triglyceride levels get increased in serum (Goldberg, 2001). The mechanism involved behind this is the loss of urinary protein that stimulates an increased LDL synthesis from the liver which causes upregulation of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA). While impaired chylomicrons and VLDL clearance is a dominant factor that increases serum triglyceride concentration (Vaziri, 2003; Vaziri, Sato, Liang, 2003). Alone or combined treatment with captopril & valsartan through the ICV route reduces the elevated level of serum cholesterol and serum triglyceride significantly. This may be due to the ability of ACE inhibitors and ARB to restore proteinuria which helps in maintaining high lipid complications in DN (Trevisan, Dodesini, Lepore, 2006).

Due to vascular endothelial dysfunction (VED) in DN reduced serum nitrite concentration was observed (Jindal, Singh, Balakumar, 2007). Serum nitrite level and kidney weight/body weight were considered as the most important marker that indicates renal insufficiency (Remmuzi, Bertani, 1990). Again alone or combined treatment with captopril and valsartan through the ICV route significantly elevates the reduced serum nitrite level. The most significant elevation in serum nitrite level is seen in combined drug therapy (captopril & valsartan) which may be due to VED restoration. However, the ratio of kidney weight/body weight in diabetic rats gets increased significantly due to the presence of renal hypertrophy which increases the weight of the kidney in both diabetic and vehicle groups (Zhang *et al*., 2015). Treatment with captopril & valsartan alone or combined therapy reduces the elevated kidney weight/body weight significantly. This may be due to a reduction of Ang-II level which causes cell proliferation & renal hypertrophy (Remuzzi *et al*., 1991).

## CONCLUSION

Based on the above discussion, it may be concluded that co-administration of valsartan and captopril attenuates diabetic nephropathy. These observed renoprotective effects may be due to the decreased central sympathetic outflow and peripheral activity of Ang II. It is the most responsible component in the RAAS pathway that causes nephropathy as it has effects like increased oxidative stress and most importantly vasoconstriction. The RAAS present in the body either in the brain or periphery controls the physiological activity of the renal function. Deletion of ACE2 is noted due to increasing Ang-II levels which lead to the development of DN. The experiments done, the analysis performed & the result obtained promotes us to rather strongly claim that nephropathy developed in the rat is due to increased secretion of Ang-II. Treatment was given with single and combined drug therapy of captopril and valsartan through the ICV route in the brain which show beneficial results in DN. The mechanism which may involve in this would be overexpression of the ACE2 level in the brain. Increased ACE2 levels in the brain may reduce the sympathetic outflow and might have decreased the peripheral activity of Ang-II which helps in the treatment of DN.

## ACKNOWLEDGEMENT

We pay immense gratitude to Prof. Anil Kumar (Punjab University, Chandigarh) and Ms. Arti Singh (Punjab University, Chandigarh) for assisting me in the handling of stereotaxic apparatus. With great pleasure & deep satisfaction, we express my sincere thanks to Shree Narayan Das Agarwal Ji (Chairman, GLA University, Mathura, U.P. India); Prof. Pradeep Mishra (Director, Institute of Pharmaceutical Research, GLA University) for their priceless support & guidance throughout research work.

## CONFLICT OF INTEREST

There is no conflict of interest.

## REFERENCES

Alberti KGMM, Zimmet P, Shaw J. Metabolic syndrome-a new world wide definition. A consensus statement from the international diabetes federation. Diabet Med. 2006;23(5):469-80.

Bakris GL, Weir MR. Angiotensin converting enzyme inhibitor associated elevation in serum creatinine. JAMA Intern Med. 2000;160:685-693.

Baltatu OC, Campos LA, Bader M. Local renin-angiotensin system & the brain: a continuous quest for knowledge. Peptides. 2011;32(5):1083-6.

Battle D, Soler MS, Ye M. ACE2 & Diabetes: ACE of ACE's. Diabetes. 2010;59:2540-2548.

Baum T, Becker FT, Suberts EJ. Attenuation of pressor responces to intracerebroventricular angiotensin-I by angiotensin converting enzyme inhibitors & their effect on systemic blood pressure in conscious rats. Life Sci. 1983;32(12):1297-1303.

Bertoncello N, Moreira RP, Arita DY, Aragao DS, Watanbe IKM, Dantas PS, et al. Diabetic nephropathy induced by increased ACE gene dosage is associated with high renal level of angiotensin (1-7) & bradykinin. J Diabetes Res. 2015;1-13.

Campbell R, Sangalli F, Perticucci E, Aros C, Viscarra C, Perna A, et al. Effect of combined ACE inhibitor & angiotensin-II antagonist treatment in human chronic nephropathies. Kidney Int. 2003;63:1094-1103.

Chawla T, Sharma D, Singh A. Role of the renin-angiotensin system in diabetic nephropathy. World J Diabetes. 2010;1(5):141-5.

Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.

Crackower MA, Sarao R, Oudit AY, Yagil C, Kozieradzki I, Scanga SE, et al. Angiotensin-converting & is an essential regulator of heart function. Am j physiol. 2002;17:822-828.

Doughue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme related carboxypeptidase (ACE2) converts angiotensin-I to angiotensin 1-9. Circ Res. 2000;87(5):E1-9.

Feng Y, Xia H, Cai Y, Halabi CM, Becker LK, Santos RA et al. Brain-selective overexpression of human angiotensin-converting enzyme type-2 attenuates neurogenic hypertension. Circ Res. 2010;106(2):373-82.

Fioretto P, Stechower CD, Mauer M. Heterogeneous nature of microalbuminuria in NIDDM: studies of endothelial function & renal structure. Diabetologia. 1998;41(2):233-6.

Giani JF, Munoz MC, Pons RA. Angiotensin (1-7) reduces proteinuria & diminishes structural damage in renal tissue of stroke-prone spontaneously hypertensive rats. Am J Physiol Renal Physiol. 2011;300(1):F272-82.

Goldberg IJ. Diabetic Dyslipidemia: Cause & Consequences. J Clin Endocrinol Metab. 2001;86(3):965-71.

Haley TJ, McCormick WG. Pharmacological effects produced by Intracerebral Infection of drugs in the conscious mouse. Brit J Pharmacol. 1957;12:12-15.

Jindal S, Singh, Balakumar P. Effect of bis(maltolato) oxovandiun (BMOV) in uric acid & sodium arsenite induced vascular endothelial dysfunction in rats. Int J Cardiol. 2008;128(3):383-91.

Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Med J Aust. 2014;7(1):45-48.

Kelly DJ, Zhang Y, Naik MG, Gilbert RE. Aliskiren, a novel renin-inhibitor, is renoprotective in a model of advanced diabetic nephropathy in rats. Diabetologia. 2007;50(11):2398-404.

Kobori H, Kamiyama M, Bernard LMH, Navar G. Cardinal role of the intrarenal renin-angiotensin system in the pathogenesis of diabetic nephropathy. J Investig Med. 2013;61:256-264.

Kushwaha S, Malpani A, Aswar UM, Bodhankar SL, Malpani A, Shivakumar SJ. Effect of different anaesthetic agents on cardiovascular parameters in male Wistar rats. Res J Pharm Biol Chem Sci. 2011;2(2):685-690.

Leehey DJ, Singh AK, Alavi N, Singh R. Role of angiotensin-II in diabetic nephropathy. Kidney Int. 2000;58(77):593-598.

Lewis EJ, Hunsicker LG, Bain RR, Rohde RD. The effect of angiotensin-converting enzyme inhibition on diabetic nephropathy. N Engl J Med. 1993;329:1456-1462.

Makaya T, Chatterjee S, Arundel P, Beuan C, Wright P. Severe hypercalcemic in diabetic ketoacidosis: a case report. Diabetes Care. 2013;36(4):1-2.

Maschio G, Alberti D, Janin G, Locatelli F, Mann JFE, Motolese M, et al. Effect of the ACE inhibitor benazepril on the progression of chronic renal insufficiency. N Engl J Med. 1996;334(15):939-45.

McKinley MJ, Allen AM, Mathai ML, May C. Brain angiotensin & body fluid homeostasis. Jpn J Physiol. 2001;51:271-289.

Mogenson CE. Microalbuminuria as a predictor of clinical diabetic nephropathy. Kidney Int. 1987;31:673-689.

Nakamura K, Shimizu S, Yanagita T, Nemoto T, Tamivchi K, Shimizu S, et al. Angiotensin-II acting on brain AT1 receptors induces adrenaline secretion & pressor responces in the rat. Sci Rep. 2014;4:1-8.

Onozato ML, Tojo A, Coto A, Fujta T, Wilcox CS. Oxidative stress & nitric oxide synthase in diabetic nephropathy: Effect of ACEI and ARB. Kidney Int. 2002;61(1):186-94.

Remmuzi G, Bertani T. Is glomerulosclerosis: a consequence of altered glomerular permeability to molecules. Kidney Int. 1990;38(3):384-94.

Remuzzi A, Petricucci E, Ruggennti P, Mosconi L, Limonta M, Remuzzi G, Angiotensin-converting enzyme inhibition improves glomerular size selectivity in IgA nephropathy. Kidney Int. 1991;39(6):1267-73.

Rosenfalck AM, Amdal T, Hislsted J, Madsbad S. Body composition adults with type 1 diabetes at onset & during the first year of insulin therapy. Diabet Med. 2002;19(5):417-23.

Ruster C, Wolf G. Renin-angiotensin aldosterone system: Pathophysiology role & Pharmacologic Inhibition. J Manag Care Pharm. 2007;13(8):S9-S20.

Santos RAS, Ferreira AJ, Braga TV, Bader M. Angiotensin-converting enzyme-2, angiotensin (1-7) & Mass receptor-new player of the renin-angiotensin system. J. Endocrinol. 2013;216 R1-R17.

Sharma V, Sharma PL. Role of different Molecular pathways in the development of diabetes-induced nephropathy. J Diabetes Metab. 2013;1-7.

Sharma VK, Kumar S, Patel HJ, Hugar S. Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rats. Int J Pharm Sci Rev Res. 2010;1(2):18-22.

Sinuani I, Averbukh Z, Gitelman I, Rapoport MJ, Sandbank J, Albeck M, et al. Mesangial cells initiate compensatory renal tubular hypertrophy via IL-10 induced TGF-β secretion: effect of the immunomodulator AS101 on this process. Am J Renal Physiol. 2006;291(2):F384-94.

Tikellis C, Bialkowski K, Pete J, Sheey K, Su Q, Johnston C, et al. ACE2 defeciency modifies renoprotection afforded by ACE inhibition in experimental diabetes. Diabetes. 2008;57:1018-1025.

Trevisan R, Dodesini AR, Lepore G. Lipids and Renal disease. J Am Soc Nephrol. 2006;17(4):S145-7.

Trinder P. Determination oxidase of glucose in blood using glucose with an alternative oxygen receptor. J Clin Pathol. 1969;22(2):158-61.

Trojacanec J, Zafirov D, Jakiovski K, Gjorgjievska K, Trojacanec P, Labaceuski N. Effect of dual RAAS blockade with candesartan & perindropril on functional renal test in STZ induced Diabetic nephropathy. Maced J Med Sci. 2013;6(3):219-226.

This is an open-access article distributed under the terms of the Creative Commons Attribution License.



Unnikrishnan RI, Rema M, Pradeep R, Deepa M, Shanthirani CS, Deepa R, et al. Prevelance & risk factor of diabetic nephropathy in an urban south Indian population-The Chennai urban rural Epidemiology study. Diabetic Care. 2007;30:2019-2024.

Vaishya R, Singh J, Lal H. Effect of Irbesartan on Streptozotocin induced diabetic nephropathy: An Interventionary study. Indian J Clin Biochem. 2008;23(2):195–197

Vaziri ND, Sato T, Liang K. Molecule mechanism of altered cholesterol metabolism in focal glomerulosclerosis. Kidney Int. 2003;63(5):1756-63.

Vaziri ND. Molecular mechanism of lipid disorder in nephritic syndrome. Kidney Int. 2003;63:1964–1976

Viberti G, Mogenson CE, Groop LC, Pauls JF. Effect of Captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus & microalbuminuria. JAMA. 1994;271(4):275-279.

Zatz R, Anderson S, Meyer TW, Durin BR, Rennke HG, Brenner BM. Lowering of arterial blood pressure limits glomerulosclerosis in rats with renal ablation & in experimental diabetes. Kidney Int Suppl. 1987;20:S123-9.

Zhang K, Meng X, Li D, Yang J, Kong J, Hao P, et al. Angiotensin (1-7) attenuates the progression of STZ-induced diabetes renal injury better than angiotensin receptor blockade. Kidney Int. 2015;87(2):359-69.

Zini S, Zaluski MCF, Chauvel E, Roques BP, Corvol P, Cortes CL. Identification of metabolic pathway of brain angiotensin-II & III using specific amino peptidase inhibitors: Predominant role of angiotensin-III in the control of vasopressin release. Proc Natl Acad Sci U S A. 1996;93(21):11968–11973.

Received for publication on 06th April 2020
Accepted for publication on 15th February 2021