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Original Article

# COMPARATIVE EFFECT OF ANGIOTENSIN II TYPE I RECEPTOR BLOCKERS ON BLOOD GLUCOSE CONCENTRATION AND OXIDATIVE STRESS IN STREPTOZOTOCIN- INDUCED DIABETIC RATS

**SNIGDHA SENAPATY, BHABAGRAHI RATH, JYOTIRMOYEE JENA, SASHI B. BISWAL**

**Department of pharmacology, V.S.S Medical College, Burla, Sambalpur, Odisha 768017, Department of Pharmacology, MGM Medical College, Kamothe, Navi Mumbai.**
**Email: snigdha.misra7@gmail.com**

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**ABSTRACT**

**Objective:** The present study was conducted to evaluate and compare the effects of ARBs (Angiotensin receptor blockers) namely telmisartan, losartan and valsartan on blood glucose concentration and oxidative stress in streptozotocin induced diabetic rats.

**Methods**: The diabetic ratswere divided into four groups of six each. They were administered test drugs such as telmisartan (5mg/kg), losartan (10mg/kg), valsartan (5mg/kg) or vehicle for 8 weeks. Blood samples were collected at 0 and 8 weeks and plasma glucose was estimated. After the treatment period, liver and kidney lipid peroxidation, reduced glutathione (GSH) and superoxide dismutase (SOD) were assayed.

**Results:** Marked hyperglycemia, elevation in tissue malondialdehyde (MDA) levels along with a reduction in SOD and GSH enzymes were observed in STZ diabetic rats. Telmisartan pretreatment reduced blood glucose levels significantly at 8 weeks whereas losartan and valsartan did not show any significant effect on blood glucose levels. All the three ARBs telmisartan, losartan and valsartan improved the oxidative stress parameters towards the normal values of control. However, telmisartan pretreatment produced significantly greater antioxidant effect compared to losartan and valsartan treated diabetic rats.

**Conclusions**: This suggests that telmisartan is superior to losartan and valsartan with regard to its glucose lowering property and antioxidant effect and may become a promising "cardio metabolic sartan" that targets diabetes and cardiovascular diseases in hypertensive patients.

**Keywords:** Angiotensin receptor blockers, Diabetes, Oxidative stress, STZ.

## INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. The incidence of cardiovascular diseases (CVD) in diabetic patients has increased up to 3 folds and is a leading cause of death worldwide. [1] Several studies have shown that hyperglycemia induces endothelial dysfunction through the generation of oxidative stress which has been suggested to be the key player in the generation of cardiovascular complications. [2] The renin-angiotensin system (RAS) plays a crucial role in circulatory homeostasis and the regulation of vascular tone. There is growing body of evidence that enhanced activation of RAS and the subsequent increase of angiotensin II & aldosterone levels contribute to changes of the insulin/ IGF-1 signaling pathway and promote the formation of ROS (reactive oxygen species) that induces endothelial dysfunction & CVD. [3] Therefore, both hyperglycemia and angiotensin II mediated action lead to oxidative stress and play a central role in the progression of diabetes and development of diabetic complication. Hypertension is common in diabetes affecting up to 60% of patients and increases the risk of complications. [4] Angiotensin II, type 1 receptor blockers (ARBs) are safe and effective drugs for the treatment of hypertension. Exogenous administration of angiotensin II receptor antagonist may be beneficial in counteracting functional changes of atherosclerosis because the rennin angiotensin system has been reported to be an important contributory factor in the path physiology of CVD. [3] Recently, telmisartan, an ARB, was found to act as a partial agonist of peroxisome proliferator- activated receptor-gamma (PPAR-γ). PPAR – γ influences the gene expression involved in carbohydrate and lipid metabolism and improves insulin resistance. [5] Moreover, there is a growing body of evidence that activators of Par exerts anti-inflammatory, anti-oxidative and anti-proliferative effects on vascular wall cells, thus decreasing the risk for atherosclerosis. [6] Thus telmisartan can function as both an ARB and as a PPAR activator.We therefore hypothesize here that telmisartan may have a greater beneficial effect in diabetes induced oxidative damage than losartan or valsartan which block angiotensin receptors but lack PPAR activation.Thus, the aim of the present study is to evaluate and compare the antihyperglycemic and antioxidant effects of telmisartan and ARBs namely losartan and valsartan in STZ induced diabetic rats.

## MATERIALS AND METHODS

### Chemicals

Telmisartan, losartan and valsartan were purchased from Glenmark Pharmaceuticals Ltd. Mumbai and STZ was purchased from Sigma Chemical Co St Louis, USA.

### Animals

Adult albino rats, of either sex, weighing between 250 – 300g were maintained under standard conditions with food and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee.

### Grouping & treatment

Animals were divided into five groups of 6 animals each and treated for 8 wks as follows:

Group – I : Normal control

Group – II : Diabetic rats treated with distilled water

Group – III : Diabetic rats treated with telmisartan ( 5 mg/kg)

Group – IV : Diabetic rats treated with losartan ( 10 mg/kg)

Group – V : Diabetic rats treated with valsartan ( 5 mg/kg )

### Induction of diabetes in experimental animals

Diabetes was induced in rats by single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight dissolved in 0.01 M (ph 4.5) citrate buffer. STZ induces diabetes within 3 days by destroying the beta cells. [7] Three days after STZ injection, rats with blood glucose levels of more than 250 mg/dl were included in the

study. Treatment with test drugs (telmisartan, losartan and valsartan) was started 5 days prior to STZ injection and continued for 8 weeks.

**Sample preparation**

The experimental period lasted for 8 weeks and at the end of the experimental period the animals were fasted overnight and sacrificed by decapitation. The tissue samples of liver and kidney were quickly removed, weighed, perfused immediately with ice cold saline (0.85% w/v NaCl) and homogenized in chilled phosphate buffer (0.1 M,ph 7.4) containing potassium chloride (1.17 %,w/v). The homogenate was centrifuged (800g, 5 min, 4ºC) to remove debris. The supernatant so obtained was centrifuged at 10,000g for 20 min at 4ºC to get postmitochondrial supernatant preparation, which was used for various biochemical assays.

**Determination of blood glucose**

Blood samples for glucose determination were obtained by puncturing retro-orbital plexus before sacrificing the animals and blood glucose level was estimated by glucose- oxidase assay method. [8]

**Biochemical assays**

**Lipid per oxidation (LPO)**

Lipid per oxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA). [9] To 1 ml of supernatant, 0.5 ml of 30% trichloroacetic acid (TCA) was added followed by 0.5 ml of 0.8% TBA. The tubes were kept in a shaking water bath for 30 min at 80ºC. After 30 min of incubation the tubes were taken out and kept in ice cold water for 10 min. These were then centrifuged at 800g for 15 min. The absorbance of supernatant was read at 540 nm at room temperature against an appropriate blank. The concentration of MDA was measured from the standard calibration curve. Lipid per oxidation was expressed as mM per 100 gm of tissue.

**Reduced glutathione (GSH) Assay**

Reduced glutathione activity was assayed according to the method of Ellman. [10] Reduced glutathione in the liver and kidney homogenate was estimated spectrophotometrically by determination of 2-nitro 5 thiobenzoic acid (yellow colour) formed as a result of reduction of DTNB (Dithiobis- 2-nitrobenzoic acid) by GSH.

**Superoxide dismutase (SOD) activity**

The SOD activity was measured according to the method used by Marklund and Marklund. [11] The enzyme activity was expressed as units/ mg protein and one unit of enzyme is defined as the enzyme activity that inhibits autoxidation of pyrogallol by 50%.

**Statistical Analysis**

Data is represented as Mean ± SEM and analyzed using one-way ANOVA followed by Tukey multiple comparison tests. P< 0.05 were considered statistically significant.

**RESULTS**

**Effect on blood glucose concentration**

Figure 1 shows blood glucose concentration in the rats at 0wks & 8wks. The STZ induced diabetic rats exhibited significant (p 0.001) elevation in the blood glucose level compared to those of normal group after 72 hrs of STZ injection. The results showed that telmisartan produced a significant (p 0.05) decrease in blood glucoseat the end of 8 wks in diabetic rats (Group III). However, there was no significant reduction in the blood glucose level in losartan and valsartan treated diabetic group (Group IV & Group V).

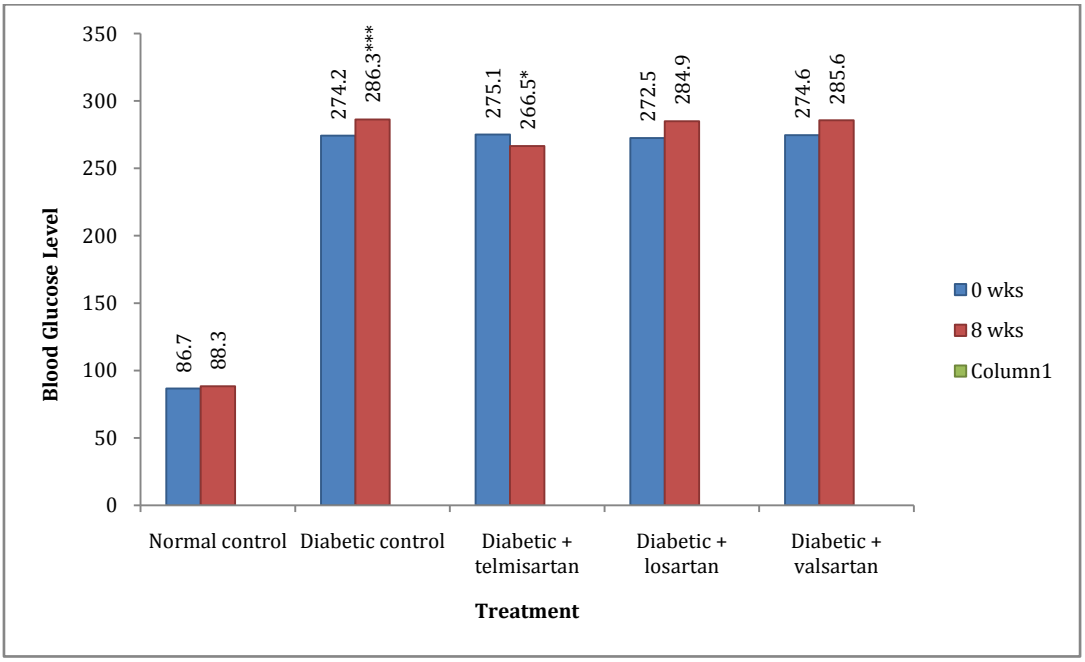


**Fig. 1: Effect of various ARBs (Angiotensin receptor blockers) on blood glucose levels in normal and diabetic treated ratsValues are expressed as mean ± SEM for 6 animals in the group. ***P < 0.001 as compared to vehicle treated control group, *P < 0.05 as compared to diabetic control group**

**Effect on Hepatic and Renal Oxidative Stress Parameters**

There was a significant (p 0.01) increase in tissue MDA level in liver and kidney in diabetic group (Group II) compared to control group (Group I). Administration of telmisartan significantly (p < 0.01) decreased MDA level in diabetic rats (Group III). Losartan and valsartan treated groups (Group IV & Group V) also produced a significant (p<0.05) decrease in diabetic rats. The effect of telmisartan on tissue MDA levels was significantly greater when compared to the effect of losartan and valsartan. There were no significant differences between the effects of losartan and valsartan on MDA levels in liver and kidney in diabetic rat [Table 1].

**Table 1: Effect of ARBs on lipid peroxidation (MDA mM /100g of tissue) of rat liver and kidney**

| Groups | Lipid peroxides (mM /100g of tissue) | |
|---|---|---|
| | Liver | Kidney |
| Normal control | 0.93 ± 0.15 | 3.36 ± 0.16 |
| Diabetic control | 1.86 ± 0.18** | 11.38 ± 0.19** |
| Diabetic + telmisartan | 0.98 ±0.17** | 5.58 ± 0.22** |
| Diabetic + Irbesartan | 1.43 ±0.27* | 8.05 ± 0.52* |
| Diabetic + Valsartan | 1.51 ± 0.29* | 8.35 ±0.67* |

**Values are mean± SEM (n =6 per group)**P < 0.01 Vs control; **P < 0.01, *P < 0.05 Vs diabetic control;**

**Table 2: Effect of ARBs on the antioxidant status of rat liver and kidney**

| Groups | Treatment | GSH ( mg/100gm) | | SOD ( units/mg) | |
|---|---|---|---|---|---|
| | | Liver | Kidney | Liver | Kidney |
| I | Control | 34.28 ± 2.47 | 30.47 ± 2.72 | 123.05 ± 4.76 | 36.28 ± 2.54 |
| II | STZ | 20.9 ± 1.95** | 16.10 ± 2.03** | 39.65 ± 4.28** | 20.66 ± 3.51** |
| III | STZ+ telmisartan | 31.79 ± 2.28** | 29.02 ± 1.24** | 91.62 ± 5.81** | 30.57 ± 2.38** |
| IV | STZ + losartan | 26.82 ± 1.76* | 22.62 ± 2.75* | 78.78 ± 6.55* | 25.65 ± 1.83* |
| V | STZ +valsartan | 25.76 ± 1.88* | 21.7 ± 2.82* | 74.50 ± 6.74* | 24.63 ± 1.64* |

Superoxide dismutase: SOD, reduced glutathione: GSH; Data are mean ± SEM (n= 6 per group) **P < 0.01 Vs control (Group I), **P < 0.01Vs diabetic control and *P < 0.05 Vs diabetic control (Group II)

**Effect on Hepatic and Renal Antioxidant Parameters**

Hepatic & renal GSH and SOD activities significantly (p <0.01) decreased in Diabetic Control when compared with normal control. Groups III, IV & V exhibited significant increase in the levels of GSH & SOD in diabetic rats. However, the increase in the levels of GSH & SOD produced by telmisartan treated diabetic rats (Group III) was significantly (p < 0.05) higher when compared to losartan and valsartan treated diabetic rats (Group IV & Group V). There were no significant differences in the effect between Group IV and Group V [Table 2].

**DISCUSSION**

This study was designed to evaluate and compare the effects of ARBs namely telmisartan, losartan & valsartan on blood glucose and oxidative stress in STZ induced diabetic rats. Diabetes mellitus in rodents is a reliable and useful model for rapid observation of the protective effects of investigated agents on diabetes- induced damage. [12] STZ injected intraperitoneally at a dose of 50mg/kg effectively induced diabetes after 72 hrs as reflected by high blood glucose levels in normal fasted rats.The hyperglycemia and diabetes were imputed to the selective destruction of pancreatic β cells that secrete insulin. [13]

The STZ diabetic rats exhibited persistent hyperglycemia which contributes to the increase in oxygen free radicals by autoxidation of glucose. [14] The reactive oxygen species in turn cause lipid per oxidation and membrane damage. [15] Diabetes increases oxidative stress in many organs, especially in the liver, and thus may play a role in the pathogenesis and progression of diabetic tissue damage. [16, 17] Lipid per oxidation is a commonly used index of increased oxidative stress and subsequent cytotoxicity.In this study, the level of MDA, an indicator of free radical generation and endproduct of lipid per oxidation, was significantly increased in the untreated diabetic rat liver and kidney.Malondialdehyde is chemically reactive if not removed from the cell by antioxidant mechanisms, and may cause cellular damage inactivation, protein and DNA damage. [18] Oxidative stress in diabetes coexists with a reduction in antioxidant power. [19] Glutathione protects the cellular system against toxic effects of lipid per oxidation and SOD enzyme scavenges the superoxide radical by converting it to $H_2O_2$ and molecule $O_2$. [20]

According to our results, a reduction in blood glucose level was observed in telmisartan treated diabetic group whereas there was no significant lowering in blood glucose in losartan and valsartan treated diabetic rats. This may be possible because telmisartan has been shown to have a structural similarity with the molecule of pioglitazone, a ligand of peroxisome proliferator- activated receptor γ, which stimulates insulin sensitivity thereby decreasing the blood glucose level independent of the action of rennin- angiotensin system. [5, 6] The study further revealed that ARBs reduced MDA levels and produced significant elevation of GSH & SOD in treated diabetic rats. This confirms the antioxidant potential of ARBs which is in accordance with previous studies which reported that Ang-II increases nicotinamide adenine dinucleotide phosphate oxidase activity, via the $AT_1Rs$, which, in turn, increases the production of reactive oxygen species (ROS). Ang-II, in combination with ROS, leads to endothelial cell dysfunction and endothelial cell apoptosis in the CV tissues through inhibition of the antiapoptotic effects of protein Bcl-2. [21] Experimental studies in both animals and humans have demonstrated that the ACE inhibitors and ARBs possess antioxidant effects through their action on the $AT_1R$ and $AT_2R$. [22] However, our results suggest that telmisartan was more effective than losartan & valsartan in reducing the oxidative stress. There is a correlation between the decrease in hyperglycemia and the reduction of oxidative stress. Telmisartan has the capacity to both activate PPARs and block angiotensin receptors. Because such compounds exert anti-inflammatory effects through multiple pathways (PPAR pathway and the angiotensin receptor pathway), they provide a superior ability to treat or prevent inflammatory diseases than PPAR activators or ARBs alone. Such compounds that activate PPARs and block angiotensin receptors are also superior to PPAR activators because unlike currently recognized PPAR activators, the compounds of the current invention do not promote or aggravate fluid retention, peripheral edema, pulmonary edema or congestive heart failure. Therefore, it was likely that telmisartan alleviated the lipid per oxidation and tissue injuries through antihyperglycemic and antioxidant enzyme activity

**CONCLUSION**

The results of present study indicate the telmisartan has a unique glucose lowering property, which was not found with other drugs tested. Also the antioxidant property of telmisartan is superior to losartan and valsartan. Therefore, ARBs while having definite beneficial effect in retarding the progression of cardiovascular diseases in general, telmisartan can be considered as the best drug for the prevention and treatment of this important complication of diabetes mellitus.

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