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Research Article

Hyperglycaemia-Induced Nephropathy is Prevented by Resveratrol and Pioglitazone Co-Administration in Type-2 Diabetic Male Wistar Rats

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Keywords:

Diabetes mellitus, Diabetic nephropathy, Hyperglycaemia, Oxidative stress, Resveratrol, Pioglitazone, Lisinopril.

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ABSTRACT

Background: Hyperglycaemia results in oxidative stress and activation of certain pathways such as that of aldose reductase, commonly observed in the development of diabetic nephropathy. The aim of this research was to evaluate the effect of resveratrol and pioglitazone co-administration in hyperglycaemia-induced nephropathy in type-2 diabetes.

Methods: Thirty (30) adult male Wistar rats were induced with type-2 diabetes through high-fat-diet and fructose feeding for six weeks, followed by a single dose of 35 mg/kg streptozotocin (STZ) injection intraperitoneally. Rats with fasting blood glucose (FBG) levels of ≥ 200 mg/dL (20) were randomly divided into 5 groups of 4 rats each. Eight (8) other apparently healthy rats received regular diet and formed groups I and II of the experiment who received 1 ml/kg distilled water and 1 ml/kg carboxymethylcellulose (CMC) respectively, group III remained untreated, group IV, V, VI and VII received 100 mg/kg resveratrol, 5 mg/kg pioglitazone, 100 mg/kg resveratrol + 5 mg/kg pioglitazone and 1 mg/kg Lisinopril respectively. All interventions were given through oral route and lasted for six weeks post STZ injection. Rats were then fasted overnight and anaesthesized with 50 mg/kg ketamine hydrochloride and 25 mg/kg diazepam. Blood was collected via cardiac puncture in plain bottles and the right kidney of each rat was homogenized for biochemical assays. Data were analyzed and expressed as mean ± standard error of mean (SEM) using one way or repeated measure analysis of variance, followed by Tukey's *post-hoc* test to compare level of significance, values of $p < 0.05$ were considered statistically significant.

Results: There was a significant decrease ($p < 0.05$) in FBG levels between the co-administration and diabetic untreated groups at weeks 8, 10 and 12. Activities of antioxidant enzymes SOD, CAT and GSH in kidney homogenate increased significantly with a corresponding statistical significant decrease in MDA concentration between co-administration and diabetic untreated groups. Also, significant decreases ($p< 0.05$) were seen in serum levels of aldose reductase and KIM-1 in the co-administration group compared to the diabetic control.

Conclusion: The outcome of this study shows that resveratrol could potentially augment the effect of anti-diabetic drugs, in this case pioglitazone, in the prevention of diabetic nephropathy development in type-2 diabetes.

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1. Introduction

Diabetes mellitus (DM) and chronic kidney disease (CKD) are now two of the fastest growing pathologies globally owing to ageing population and increased occurrence of many interrelated comorbidities such as hypertension, obesity and atherosclerosis (Quiroga *et al*., 2015). Type-2 DM occur due to widespread resistance to insulin, giving rise to a hyperglycaemic environment that disrupts metabolic and haemodynamic homeostasis, leading to the development of diffuse cellular abnormalities (Grieve *et al*., 2017). Diabetes causes various organ complications and the kidneys are the major target organs, making it the major cause of end-stage kidney disease in developed and developing countries (Wada *et al*., 2018).

Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus and ischemic diseases. Oxidative stress has been tagged as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA (Yoshikawa and Naito, 2002). Renal oxidative stress is often a result of upregulation of pro-oxidant enzyme-induced ROS production and accompanying exhaustion of antioxidants (Jha *et al.*, 2016).

Among the various pathways of diabetic complications, the polyol pathway plays an important role in the development of diabetic kidney disease (DKD). It has been suggested that the polyol pathway generates not only osmotic stress, but also hyperglycaemic oxidative stress in renal tissue (Keiichiro *et al*., 2020). Both increased aldose reductase (AR) activity and oxidative stress have been implicated in the pathogenesis of diabetic nephropathy (DN) (Drel *et al*., 2006). Severe hyperglycaemia, kidney ischemia or hypoxia promotes increase in kidney injury molecule 1 (KIM-1) concentration in the urine and blood serum. Chronic KIM-1 expression can promote further tubulointerstitial inflammation and hypoxia, further inducing KIM-1 expression that results in chronic kidney disease (Krievina *et al*., 2016).

Almost all the emerging strategies and investigations carried out to retard the progress of DN only slow down the rate at which kidney function is lost but unable to reverse or prevent it (Lindblom *et al.*, 2015). Phytochemicals from dietary or medicinal plant sources have been shown to modulate multiple cellular signalling pathways with little to no toxicity (Sethi *et al*., 2017). Several studies have shown various health benefits of resveratrol in prevention and treatment of various diseases including glycaemic control in subjects with diabetes (Huang *et al*., 2019). Thiazolidinediones, including pioglitazone are synthetic agonists of PPAR-γ and have been shown to improve glycaemic control in type-2 diabetic patients (Matsushita *et al*., 2011). They have also been reported to render direct renoprotective effects (Agrawal *et al*., 2011). This study focused on hyperglycaemia lowering effect of resveratrol and pioglitazone co-administration and the role they could play in mitigating against oxidative stress, upregulation of aldose reductase activity and high TNF-alpha concentrations in hyperglycaemia-induced nephropathy in type-2 diabetic male Wistar rats.

2. Materials and Methods

2.1 Equipment, drugs and reagents

Digital glucometer (Accu-Chek Active Advantage, Roche Diagnostics, Germany), simas margarine, 2 mL and 5 mL syringes and needles, weighing balance, cages, drinking bottles, measuring cylinder and conical flask, dissecting kit and tray. Distilled water, D-Fructose (Guangdong China, LOT: 20160812), Streptozotocin (MP Biomedicals M 3219k, France), 0.1M citrate-buffered saline (pH 4.5), carboxymethylcellulose, Trans-resveratrol (MR180519, Megaresveratrol, Danbury), pioglitazone (Micro Laboratories LTD., POHH0014, India), lisinopril (Impulse pharma PVT.LTD, 401506, India), ketamine hydrochloride, diazepam, Aldose reductase and TNF-alpha ELISA kits (Shanghai Coon Koon Biotech. Co. LTD., CK-bio-14155, China).

2.2 Ethical consideration

Ethical approval on guidelines for care and use of laboratory animals in scientific research was obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2021/133).

2.3 Experimental animals

Forty (40) adult male Wistar rats weighing between 120 to 180 g were sourced from the

Animal house of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria where the experiment was carried out. Rats were also fed standard diet and water as necessary.

2.4 Induction of type-2 diabetes mellitus

Type-2 diabetes was induced in 30 rats by feeding them high fat diet (HFD) (Normal feed + simas® margarine in the ratio 10:1) and 20 % fructose solution as drinking water for six (6) weeks (Okoduwa *et al*., 2017). The rats where then fasted overnight and given a single intraperitoneal injection of streptozotocin (STZ) at a dose of 35 mg/kg diluted in 0.1 M citrate-buffered saline (pH 4.5). FBG levels of the rats that survived were checked 3 days post STZ injection and confirmed 7 days after that to validate diabetes, rats with FBG levels ≥ 200 mg/dL (20) were considered diabetic according to Okoduwa *et al*. (2017) with some modifications.

2.5 Animal grouping

Animals were grouped into seven (7) groups of four (4) animals each (n=4). All drugs were administered orally, daily for six (6) weeks.

Group I: Normal Control given 1 ml/kg Distilled water

Group II: Normoglycaemic + Carboxymethylcellulose (CMC) 1 mL/kg

Group III: Negative control + Carboxymethylcellulose (CMC) 1 mL/kg

Group IV: Diabetic + Resveratrol 100 mg/kg (Nwasor *et al*., 2018)

Group V: Diabetic + Pioglitazone 5 mg/kg (Khodeer *et al*., 2015)

Group VI: Diabetic + Resveratrol 100 mg/kg + Pioglitazone 5 mg/kg

Group VII: Diabetic + Lisinopril 1 mg/kg (Balakumar *et al*., 2010).

2.6 Blood glucose level determination

Fasting blood glucose levels of rats were measured every two weeks to monitor the progress of blood glucose changes throughout the experimental period using a standard Accu-Chek® Active glucometer. The tail of the rat was slightly cut using a scissors to expose the vein and a drop of blood was put on the glucometer strip attached to the glucometer. The reading was recorded in mg/dL for each rat.

2.7 Body weight measurement

Body weights of the experimental rats were measured every two weeks for welfare monitoring from the start of the experiment to the end using a digital weighing balance. The weights were recorded accordingly in grams (gm) and analyzed.

2.8 Blood sample collection

Following six (6) weeks intervention period, the rats were fasted overnight and then anaesthesized with 50 mg/kg ketamine hydrochloride and 25 mg/kg diazepam. 5 mL of blood was collected via cardiac puncture in plain bottles and centrifuged at 3000 x g for 10 minutes to obtain serum for biochemical assays.

2.9 Determination of oxidative status of hyperglycaemic Wistar rats

Assay of biomarkers of oxidative stress in right kidney homogenate of both normal and hyperglycaemic rats was done by colorimetric method thus;

Superoxide dismutase activity was evaluated according to the method of Fridovich, 1989, catalase activity according to the method of Aebi, 1984, reduced glutathione levels according to the method of Mannervic, 1999 and malondialdehyde (MDA) concentrations as an index of lipid peroxidation according to the method of Niehaus and Samuelson, 1968.

2.10 Determination of serum Aldose Reductase activity and Kidney Injury Molecule-1 (KIM-1) concentration in hyperglycaemic Wistar rats

Activity of aldose reductase and concentration of KIM-1 as specific markers of DN development were determined using specie-specific ELISA kits according to the manufacturer's instructions as described by Abubakar *et al*. (2021) and Sabbisetti *et al*. (2014) respectively.

2.11 Statistical analysis

Data collected were expressed as mean ± standard error of mean (SEM). Data were also analyzed using one way or repeated measure analysis of variance (ANOVA) as appropriate using SPSS version 23, followed by Tukey's *post-hoc* test to compare the level of significance between the groups, values of $p < 0.05$ were considered statistically significant.

3. Results

3.1 Fasting Blood Glucose Levels of Hyperglycemic Wistar Rats

Statistical significant decrease (p = 0.000) was observed in the co-administration group compared to the diabetic control group on weeks 8 (p =0.000), 10 (p =0.001) and 12 (p =0.000). Conversely, there was a significant increase in FBG in the diabetic control group compared to the normal control group on these weeks (p = 0.000)

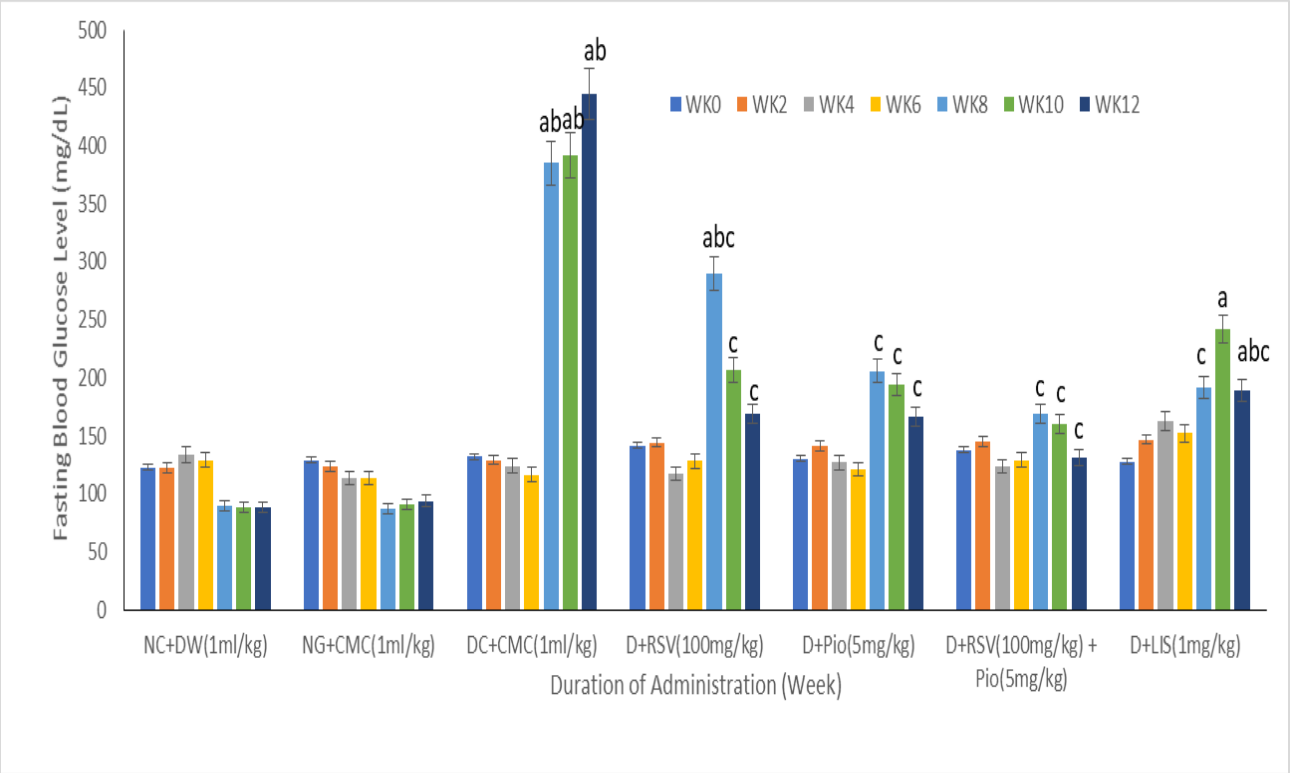

Figure 1: Bi-weekly fasting blood glucose levels of Wistar rats given co-administration of resveratrol and pioglitazone for six weeks. Values are presented as mean ± SEM; n=4: $p < 0.05$ = significant. Superscripts: a= statistically significant compared to NC+DW, b= compared to NG+CMC and c= compared to DC+CMC.

NC+DW = Normal control treated with Distilled water 1 ml/kg, NG+CMC(1ml/kg) = Normoglycemic rats treated with Carboxylmethylcellulose 1 ml/kg, DC+CMC (1ml/kg) = Diabetic rats treated with kg)+Pio (5 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol and 5 mg/kg of Pioglitazone Carboxylmethylcellulose 1 ml/kg, D+RSV (100 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol, D+Pio (5 mg/kg) = Diabetic rats treated with 5 mg/kg of Pioglitazone, D+RSV (100 mg/ and D+LIS (1 mg/kg) = Diabetic rats treated with Lisinopril 1 mg/kg

3.2 Body Weight Distribution of Hyperglycaemic male Wistar Rats

Diabetic control group showed a statistical significant difference on weeks 8 ($p = 0.007$), 10 ($p = 0.001$) and 12 ($p = 0.000$) compared to the normal control group. The co-administration group didn't show any significant difference ($p > 0.05$) compared to the diabetic control.

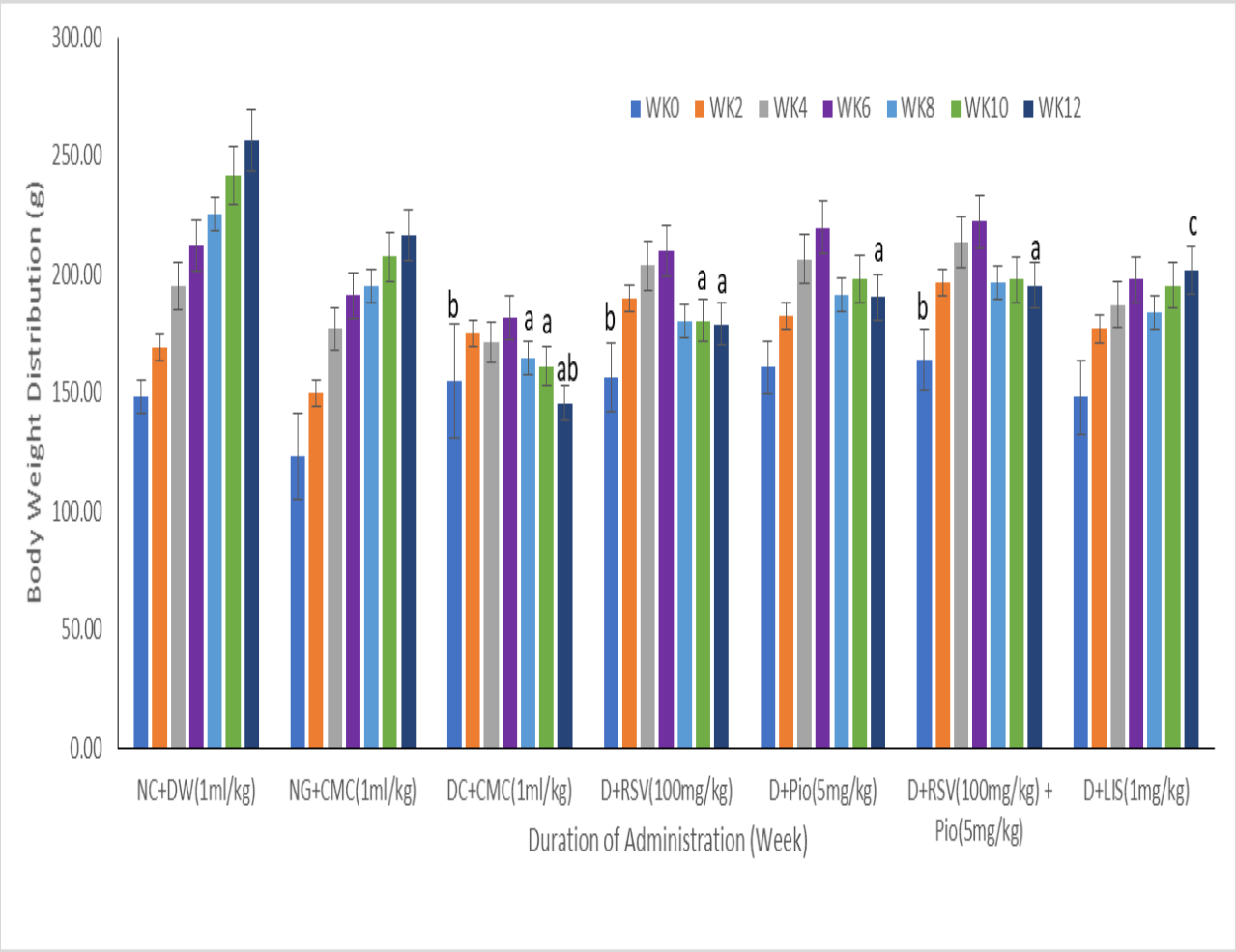

Figure 2: Bi-weekly body weight distribution of Wistar rats given co-administration of resveratrol and pioglitazone. Values are presented as mean ± SEM; n=4: $p < 0.05$ = significant. Superscripts: a= statistically significant compared to NC+DW, b= compared to NG+CMC and c= compared to DC+CMC.

NC+DW = Normal control treated with Distilled water 1 ml/kg, NG+CMC(1ml/kg) = Normoglycemic rats treated with Carboxylmethylcellulose 1 ml/kg, DC+CMC (1ml/kg) = Diabetic rats treated with kg)+Pio (5 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol and 5 mg/kg of Pioglitazone Carboxylmethylcellulose 1 ml/kg, D+RSV (100 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol, D+Pio (5 mg/kg) = Diabetic rats treated with 5 mg/kg of Pioglitazone, D+RSV (100 mg/ and D+LIS (1 mg/kg) = Diabetic rats treated with Lisinopril 1 mg/kg

3.3. Effect of six weeks (42 days) daily co-administration of resveratrol (100 mg/kg) and pioglitazone (5 mg/kg) on antioxidant enzymes and lipid peroxidation in hyperglycaemic male Wistar rats

Compared to the diabetic control, the co-administration group showed statistical significant increases in values of SOD ($p = 0.037$) and CAT ($p = 0.000$) due to upregulation of these enzymes (Table1). Also, the diabetic control group revealed significant decreases ($p = 0.000$ and 0.001) in SOD and GSH levels compared to the normal control group. A statistical significant decrease was also observed in MDA levels ($p = 0.000$) of co-administration group compared to the diabetic control group.

Table 1: Activities of antioxidant enzymes and level of lipid peroxidation in kidney homogenate of hyperglycaemic Wistar rats treated with a combination of resveratrol (100 mg/kg) and pioglitazone (5 mg/kg).

Groups	SOD (u/mg)	CAT (u/mg)	GSH (ug/mg)	MDA (umol/mg)
NC+DW(1ml/kg)	36±3.20*	31±3.49	32±1.92*	126±2.24
NG+CMC(1ml/kg)	15±1.72*	25±1.60	29±2.33*	135±2.82*
DC+CMC(1ml/kg)	8±0.48$^{\#}$	23±0.73	16±0.29$^{\#}$	354±9.13$^{\#}$
D+RSV(100mg/kg)	23±1.70*	33±2.10*	17±1.14	115±6.31*
D+Pio(5mg/kg)	36±1.99*	43±2.17*	36±3.78*	101±2.91*
D+RSV(100mg/kg) + Pio(5mg/kg)	17±0.44*	40±0.76*	20±1.38	91±1.61*
D+LIS(1mg/kg)	14±0.86*	39±2.51*	23±3.35$^{\#,*}$	139±4.32*

Values are presented as mean ± SEM; n=4: $p < 0.05$ = significant. Superscripts: $^{\#}$ = significant difference when compared to NC group, and *= significant difference when compared to DC group.

NC+DW = Normal control treated with Distilled water 1 ml/kg, NG+CMC(1ml/kg) = Normoglycemic rats treated with Carboxylmethylcellulose 1 ml/kg, DC+CMC (1ml/kg) = Diabetic rats treated with kg)+Pio (5 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol and 5 mg/kg of Pioglitazone Carboxylmethylcellulose 1 ml/kg, D+RSV (100 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol, D+Pio (5 mg/kg) = Diabetic rats treated with 5 mg/kg of Pioglitazone, D+RSV (100 mg/ and D+LIS (1 mg/kg) = Diabetic rats treated with Lisinopril 1 mg/kg

3.4 Effect of six weeks (42 days) daily co-administration of resveratrol (100 mg/kg) and pioglitazone (5 mg/kg) on KIM-1 concentration in hyperglycaemic male Wistar rats

Co-administration of resveratrol and pioglitazone caused a statistical significant decrease (p = 0.000) compared to diabetic control in levels of KIM-1. Diabetic control group showed statistical significant increase (p = 0.000) compared to normal control.

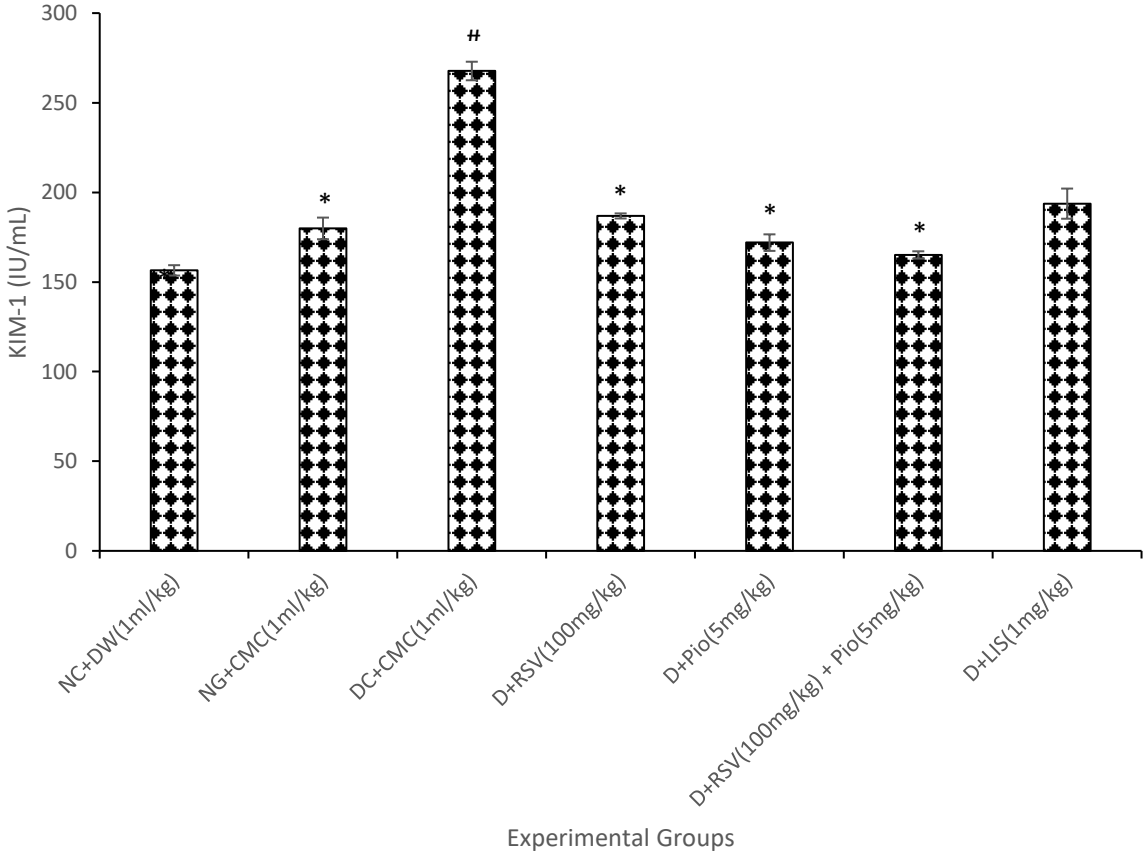

Figure 3: KIM-1 concentration in serum of Wistar rats treated with a combination of resveratrol and pioglitazone for six weeks. Values are presented as mean ± SEM; n=4: $p < 0.05$ = significant. Superscripts: $^{\#}$ = significant difference when compared to NC group, and * = significant difference when compared to DC group.

NC+DW = Normal control treated with Distilled water 1 ml/kg, NG+CMC(1ml/kg) = Normoglycemic rats treated with Carboxylmethylcellulose 1 ml/kg, DC+CMC (1ml/kg) = Diabetic rats treated with kg)+Pio (5 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol and 5 mg/kg of Pioglitazone Carboxylmethylcellulose 1 ml/kg, D+RSV (100 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol, D+Pio (5 mg/kg) = Diabetic rats treated with 5 mg/kg of Pioglitazone, D+RSV (100 mg/ and D+LIS (1 mg/kg) = Diabetic rats treated with Lisinopril 1 mg/kg

3.5 Effect of six weeks (42 days) daily co-administration of resveratrol (100 mg/kg) and pioglitazone (5 mg/kg) on aldose reductase activity in hyperglycaemic male Wistar rats

Co-administration of resveratrol and pioglitazone lead to a statistical significant decrease (p = 0.000) compared to diabetic control in levels of aldose reductase.

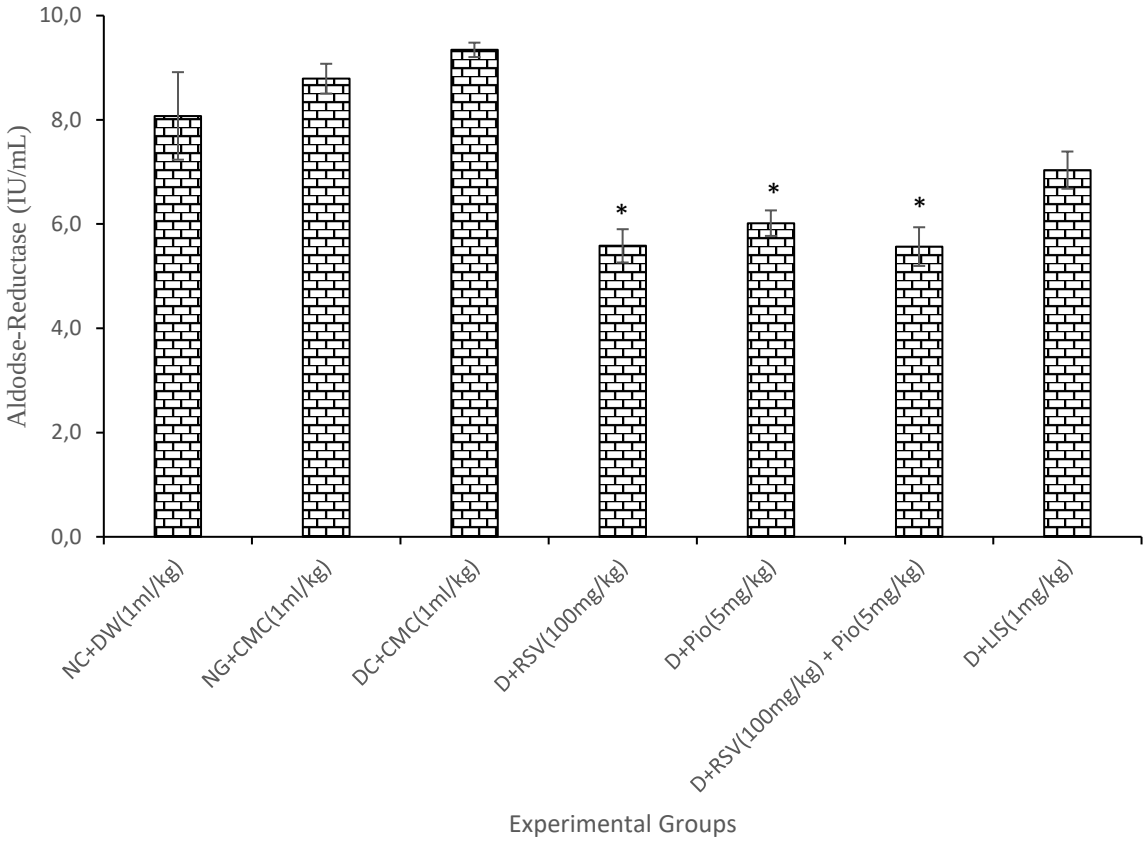

Figure 4: Levels of aldose reductase activity in serum of rats treated with co-administration of resveratrol and pioglitazone. Values are presented as mean ± SEM; n=4: $p < 0.05$ = significant. Superscripts: $^{\#}$ = significant difference when compared to NC group, and * = significant difference when compared to DC group

NC+DW = Normal control treated with Distilled water 1 ml/kg, NG+CMC(1ml/kg) = Normoglycemic rats treated with Carboxylmethylcellulose 1 ml/kg, DC+CMC (1ml/kg) = Diabetic rats treated with Carboxylmethylcellulose 1 ml/kg, D+RSV (100 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol, D+Pio (5 mg/kg) = Diabetic rats treated with 5 mg/kg of Pioglitazone, D+RSV (100 mg/kg)+Pio (5 mg/kg) = Diabetic rats treated with 100 mg/kg of Resveratrol and 5 mg/kg of Pioglitazone and D+LIS (1 mg/kg) = Diabetic rats treated with Lisinopril 1 mg/kg

4. Discussion

Diabetic nephropathy eventually develops in about 40% of DM patients (Kitada *et al*., 2016). The increasing prevalence of DN equates with the significant rise in prevalence of diabetes globally (Alicic *et al.*, 2017). This study focused on the possible preventive effects of co-administration of resveratrol and pioglitazone on hyperglcaemia and other markers of nephropathy in diabetic male Wistar rats.

Fasting blood glucose (FBG) levels of both single and co-administration groups showed a statistical significant decrease through weeks 8, 10 and 12 compared to the diabetic control group. The same outcome was observed in the lisinopril-treated group at weeks 8 and 10. Conversely, significant increases in FBG levels

was noted in weeks 8, 10 and 12 in diabetic control group compared with the normal and CMC only groups. This outcome agrees with a study done by Jimoh *et al*., in 2021 in which glycaemic profile of cholesterol-fed rabbits significantly decreased following resveratrol administration. A study conducted by Zhang *et al*., (2021) also supports this research outcome, fasting blood glucose of rats with gestational diabetes treated with resveratrol reduced significantly compared with the gestational diabetes untreated group. It is noteworthy that the co-administration does not cause hypoglycaemia, making it safe as seen in the outcome of our study. Furthermore, it reiterates the claim of resveratrol's antihyperglycaemic effect through stabilizing blood glucose levels and augmenting the action of pioglitazone in the process.

Also, outcome of co-administration of resveratrol and pioglitazone revealed a significant increase in body weights only in week 12 compared to normal control, the same outcome was observed for single administrations at week 12. However, decreases in body weights were observed in the diabetic control group as compared to the normal control group through weeks 8, 10 and 12. In diabetic rats, the body mass and weight of rats decreases as a result of insulin deficiency, and reduction in glucose transport into cells such as myocytes (Ziamajidia *et al*., 2016).

From the results obtained, the co-administration groups demonstrated significant increases in SOD and CAT and GSH activities when compared to the untreated diabetic groups in each case. This is signifying a possible role of resveratrol in free radical inactivation and in the antioxidant defense of the kidneys in the pursuit to hinder oxidative stress. It also goes to confirm that pioglitazone improves endothelial function by suppressing oxidative stress via increased superoxide dismutase activity and decreased NAD(P)H oxidase activity and also decreased ET-1 levels, which might be attributable to the inhibition of the transcription factor activator protein-1 (AP-1) signaling (Matsumoto *et al*., 2007). These results are consistent with reductions in oxidative stress biomarkers found in other studies, where resveratrol or pioglitazone treatment greatly ameliorated antioxidant enzyme activities and prevented the rise in lipid peroxides in tissue and blood cells of diabetic animals (Schmatz *et al*., 2012; Gupta *et al*., 2014). The result acquired also showed a significant decrease ($p < 0.05$) in MDA across all treatment groups, most importantly those that received co-administration of resveratrol (100mg/kg) and pioglitazone (5mg/kg), and the group treated with lisonopril at a dose of 1 mg/kg when compared to the diabetic control group. These results are in agreement with several studies that have reported an increase in biomarker activity in kidneys of animals with experimental diabetes (Palsamy and Subramanian, 2010). The decrease in MDA activity in the group treated with resveratrol signifies the antioxidant effect and membrane stability of resveratrol. This agrees with a study by Duarte-Vazquez *et al*. (2018) where co-administration of resveratrol and pioglitazone significanty reduced levels of haemoglobin A1c, insulin and glycosuria in diabetic mice.

Results from this study reveal statistical significant decreases in levels of kidney injury molecule-1 in all treatment groups compared to the diabetic untreated group, except the lisinopril-treated group whose decrease was not significant. This could be due to the antioxidant, anti-inflammatory and nephroprotective effect of resveratrol and pioglitazone, these help in restoration of endothelial and tubular integrity by keeping epithelial cells of the proximal convoluted tube intact and not compromised by persistent hyperglycaemia. This is in agreement with a study by Krievina *et al* in 2016 where increased ectopic adipose tissue storage in human participants lead to a statistical significant increase in serum KIM-1 levels. A prospective study by Li *et al*., (2016) also found that circulating KIM-1 levels were inversely proportional to estimated glomerular filtration (eGFR) in patients receiving long-term adefovir therapy in chronic hepatitis B infection. There was a significant increase ($p< 0.05$) in serum KIM-1 level of the diabetic control group compared with the normal control group due to persistent hyperglycaemia. Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein, highly expressed in epithelial cells in damaged regions of the renal proximal tubule. In both animal models and humans, urinary KIM-1 has been shown to be upregulated during acute kidney injury (AKI)

caused by toxicity, ischemia, sepsis, and renal cell carcinoma (Li *et al*., 2016). Studies have shown that KIM-1 may be released into the circulation as a result of altered microvascular permeability in humans and rats, and identified KIM-1 as a blood biomarker that specifically reflects injury to the proximal tubule of the kidney (Li *et al*., 2016; Krievina *et al*., 2016).

Outcome of aldose reductase assay showed a significant ($p < 0.05$) decrease in AR activity in the resveratrol only, pioglitazone only and co-administration groups, compared with the diabetic control group. This is in agreement with studies by Ciddi and Dodda in 2014 where resveratrol treatment in Wistar rats resulted in decreased proteinuria which was achieved through inhibition of AR. However, decreased AR activity in the lisinopril-treated group was not statistically significant. Also, our study revealed a statistical significant difference between the diabetic control and normal control groups. The aldose reductase assay showed a significant ($p < 0.05$) decrease in AR activity in the resveratrol only, pioglitazone only and co-administration groups, compared with the diabetic control group. This is in agreement with studies by Ciddi and Dodda in 2014 where resveratrol treatment in Wistar rats resulted in decreased proteinuria which was achieved through inhibition of AR. The outcome of this study could also be due to NADPH sparing potential of resveratrol leading to lower concentration of aldose reductase in the treatment groups. Intracellular hyperglycemia activates the polyol pathway, which is one of the pathogenic pathways involved in diabetic microvascular complications. AR is the key enzyme that catalyzes nicotinamide adenine dinucleotide phosphate (NADPH) in the polyol pathway, which eventually leads to the reduction of glucose to sorbitol (Sung *et al*., 2010). Because AR utilizes NADPH to catalyse glucose reduction, it was suggested that tissue injury associated with high glucose may be due, in part, to increased NADPH utilization by the reduction of glucose by AR. At normal glucose levels, AR-catalyzed reduction represents less than 3% of total glucose utilization, whereas in the presence of high glucose, more than 30% of the glucose is used by AR, suggesting that the profound increase in the AR-catalyzed reductive pathway may impose a significant strain on NADPH supply. Because NADPH is used for several critical reductive metabolic steps, such as the detoxification of ROS and hydroperoxides especially by the glutathione reductase/glutathione peroxidase system, a large drain on the NADPH pool could compromise the ability of the cell to protect itself from oxidative stress (Srivastava *et al*., 2005).

5. Conclusion

The co-administration of resveratrol and pioglitazone has anti-hyperglycaemic potential and also improves weight loss in diabetic rats. Based on the outcome of this study, it prevented diabetic nephropathy through improvement of oxidative status by up-regulating antioxidant enzymes and decreasing lipid peroxidation in the cells, reduction in KIM-1 concentration and dampening the activity of aldose reductase in hyperglycaemic male Wistar rats. Therefore, the co-administration of resveratrol and pioglitazone could play a role in the prevention of diabetes mellitus progression into diabetic nephropathy.

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