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Research Article

Cellgevity® Supplement Stalls Diabetic Renal Dysfunction in Male Rats

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ABSTRACT

Chronic excessive ROS formation instigates oxidative stress, inflammation and the inhibition of vital physiological processes, including renal Na^+/K^+-ATPase activity, thereby facilitating the progression of diabetic kidney damage. This study investigated the therapeutic impact of Cellgevity® (a poly-antioxidant supplement) against streptotozin-induced diabetic kidney dysfunction in male rats. Twenty-eight matured male rats randomised into – Control group, Diabetes-Untreated, Diabetes-Treated 1 and Diabetes-Treated 2 groups. Daily oral treatment of the Diabetes-Treated groups with therapeutic doses of Cellgevity® suspension in distilled water (25 mg/kg and 40 mg/kg BW respectively) was conducted for 30 days, while the control and Diabetic-Untreated groups received distilled water (placebo). Results show that Cellgevity® reduced kidney lipid peroxidation, prevented kidney enlargement and renal TNF-α and nitrite accumulation, and increased renal Na^+/K^+-ATPase activity compared to the untreated diabetic group. The Cellgevity® treatment also increased the actions of renal glutathione peroxidase, superoxide dismutase, and catalase by at least 70% compared to the untreated diabetic group. The serum levels of creatinine, blood urea nitrogen, HCO_3, Na^+ and K^+ of the treated diabetic groups were also significantly normalised to the levels of the control group. The results demonstrate the anti-oxidative-nitrosative and anti-inflammation impact of Cellgevity® against diabetic renal dysfunction. The result presents a good incentive for anti-oxidant supplements in the management of diabetes and its complications.

Keywords: *Diabetes, Kidney dysfunction, Oxidative stress, Cellgevity® Na^+/K^+-ATPase, Inflammation*

INTRODUCTION

Aside macrovascular dysfunction, diabetic hyperglycemia also facilitates microvascular injuries, particularly to the eye and kidney, manifested as diabetic retinopathy and nephropathy, respectively (Brownlee, 2001; Forbes, *et al.*, 2008; Kashihara *et al.*, 2010; Faselis, C., 2020). Diabetic nephropathy is a leading cause of chronic kidney disease, having high morbidity and mortality rates (Kashihara *et al.*, 2010; Tiwari *et al.*, 2013). The interaction of hyperglycemia and oxidative stress and the mechanisms by which they affect renal redox homeostasis and ultimately contribute to the development of diabetic nephropathy involves multiple pathways of ROS generation. These pathways include; the mitochondria, polyol pathway, NADPH oxidase, pentose phosphate pathway, nitric oxide synthase, xanthine oxidoreductase, advance-glycation end products and inflammation (Bohlender *et al.*, 2005; Navarro and Mora, 2005; Forbes *et al.*, 2008; Kashihara *et al.*, 2010). Also, the progression of diabetic complications occurs with decreased Na^+/K^+-ATPase activities within several tissues (Dufayet De La Tour *et al.*, 1998; Djemli-Shipkolye *et al.*, 2001; Koc *et al.*, 2003; Vague *et al.*, 2004). Na^+/K^+-ATPase is a transmembrane protein that is primarily responsible for the active transport of sodium and potassium using the energy from the hydrolysis of ATP; it helps to maintain the electrical potential necessary for secondary active transport of solutes (metabolites and nutrients) and for neuronal and muscle excitability (Djemli-Shipkolye *et al.*, 2001; Kaplan, 2002; Iannello *et al.*, 2007; Iwalokun and Iwalokun, 2007). Several hyperglycemia-mediated events such as; protein

glycation, diacylglycerol (DAG) activation of protein kinase C cause the inhibition of Na^+/K^+-ATPase in diabetic kidneys. ROS-induced membrane lipid dysfunction also affects membrane fluidity, protein (Na^+/K^+-ATPase) anchorage and activity (Djemli-Shipkolye *et al.*, 2001; Vague *et al.*, 2004; Bohlender *et al.*, 2005). Notably, the decrease in erythrocyte Na^+/K^+-ATPase activity might cause increased cytosolic osmolality, cell turgidity, reduced flexibility and restricted passage through narrow blood vessels, causing microvascular damages (Koc *et al.*, 2003; Zadhoush *et al*., 2015). The aforementioned, together with other effects of diabetes on the kidney, facilitate the progression of diabetic nephropathy (Galle, 2001; Ha and Lee, 2001; Singh *et al*., 2011). Furthermore, the depletion of renal glutathione (GSH) causing mitochondrial oxidative stress facilitates the progression of diabetic nephropathies; thus, mitochondrial glutathione redox homeostasis is essential in mitochondrial adaptability to redox imbalances associated with diabetic nephropathy (Forbes *et al.*, 2008; Coughlan *et al.*, 2009; Lash, 2015; Lutchmansingh *et al.*, 2018).

By improving redox balance, energy metabolism, inflammation control, ROS sequestering, and improved enzyme synthesis, anti-oxidant therapies are shown to be potent interventions in diabetes management (Ueno *et al.*, 2002; Kataya *et al.*, 2011; Lee *et al.*, 2017). Cellgevity® is advertised for glutathione enhancement. It is a poly-antioxidant mixture containing vitamin C, selenium, alpha-lipoic acid, broccoli seed extract, curcumin, resveratrol, grape seed extract, quercetin, milk thistle seed extract, Cordyceps, black pepper, aloe leaf and D-ribose-L-cysteine (glutathione precursor molecules) (Awodele *et al.*, 2018; Amponsah *et al.*, 2020). Reports have highlighted the therapeutic influence of many components of Cellgevity® on diabetes. Treatment with D-Ribose-L-Cysteine protects against reproductive dysfunction, hyperglycemia and hyperlipidemia in diabetic rats (Aderemi *et al.*, 2017). The effects of glutathione, resveratrol, alpha-lipoate, selenium, quercetin and vitamin C are also reported (Golbidi *et al.*, 2011; Sekhar *et al.*, 2011; Szkudelski and Szkudelska, 2011; Febiyanto *et al.*, 2017; Yang and Kang, 2018; Mason *et al.*, 2021). However, no report has shown the combined effect of all these anti-oxidants on diabetes-induced kidney complications. We earlier reported the positive impact of Cellgevity® on diabetic liver disruptions in rats (Ogunlabi *et al.*, 2021). Here we report the effect of this anti-oxidant amalgam formulation on STZ-diabetes-induced kidney oxidative stress parameters, TNF-α and nitrite levels (markers of inflammation), Na^+/K^+-ATPase activity and serum markers of kidney function (blood urea nitrogen and creatinine).

MATERIALS AND METHOD

Chemicals and reagents

Cellgevity® is from Max International, (USA), (STZ) ($C_8H_{15}N_3O_7$, MW= 265.22**)** from Sigma Aldrich (USA). The assay kit for the determination of HCO_3, Na^+ and K^+ were from Randox® while kits for Glutathione peroxidase (GPx) activity, TNF-α, blood urea nitrogen (BUN) and creatinine were from Abcam® (UK). All other chemicals and reagents used were of analytical grade.

Experimental design

The animal experiment was performed following the guidelines approved by the Research and Ethics Committee of Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Nigeria (Ref No: OOU/EC/2019-0136) in conformity with the guide for the care and use of laboratory animals - U. S. National Institutes of Health Publication (Guid. Care Use Lab. Anim., 1996) (Research, 1996).

Twenty-eight (28) adult (10 weeks old) male Wistar rats (180–220 g) were housed in individual standard cages (with wood shavings as the bedding) under standard laboratory conditions. The rats had generous access to feed and water. Also, efforts were made to minimise animal suffering.

Insulin resistance was first induced in the rats (except the seven rats assigned to the control group) by three weeks of feeding with high sucrose diet (60% w/w) (Santuré *et al.*, 2002). Afterwards, diabetes was induced in the rats via an intraperitoneal injection of streptozotocin (55 mg/kg) (in 0.1 M citrate buffer pH4.2) (Konrad *et al.*, 2001). Seventy two (72) hrs after the injection, diabetic rats showing hyperglycemia (FBG>250mg/dl) were randomised into three groups of seven (7) rats each: DM-untreated (distilled water placebo), DM-treated 1 (Cellgevity® 25 mg/kg), and DM-treated 2 (Cellgevity® 40 mg/kg). We selected the dosage of Cellgevity® based on the recommended human dosage (Awodele *et al.*, 2018). Daily, oral treatments were given for 30 days. Distilled water suspension of Cellgevity® was prepared daily and given corresponding to the weight of the rats.

Animal sacrifice and tissue preparation

After the final treatment, the rats were fasted overnight and sacrificed under deep anaesthesia with diethyl ether (2%). The blood sample was taken by cardiac puncture into plane bottles and centrifuged at 4000 rpm for 5 min to obtain the serum, stored at -20ºC for further biochemical analysis.

The two kidneys were quickly excised, weighed, sliced and rinsed in ice-cold normal saline. It was homogenised in ice-cold 10 volumes (w/v) of 0.1 M phosphate-buffered saline, pH-7.4. The homogenate was centrifuged for 20 min at 15000 rpm 4ºC to remove tissue debris and organelle fractions (Kuhad and Chopra, 2007). The supernatant was

collected and immediately stored at -20°C and later used for biochemical analysis.

Preparation of kidney tissue for Na/K-ATPase activity

One gram of the kidney tissue was quickly minced and homogenised in 10 ml of ice-cold homogenising buffer (0.25 M sucrose, 1.25 mM EGTA, and 10 mM Tris, pH 7.0) using a Teflon-glass Elvehjem-Potter homogeniser. The homogenate was further diluted with 5 volumes of the homogenising buffer and was filtered through gauze. The filtrate was stored at -20ºC and used to measure Na^+/K^+-ATPase activity (Ismail-Beigi and Edelman, 1971).

Biochemical Tests

Measurement of Fasting blood glucose (FBG)

The animal blood glucose was monitored weekly after overnight fasting using the AccuChek Active© glucometer and glucose strips with blood obtained by tail vein puncture.

Measurement of Na^+/K^+-ATPase activity in kidney homogenate

The Na^+/K^+-ATPase activity in kidney tissue homogenate was assayed as previously described (Ismail-Beigi and Edelman, 1971). Tissue homogenate (0.1 ml) was equilibrated at 37° C for 5 min and mixed with 0.9 ml of the reaction medium containing; 25 mmol/l TRIS, 12.5 mmol/l K^+, 137.5 mmol/l Cl, 5 mmol/l Mg^{2+}, 5 mmol/l ATP, 115 mmol/l Na^+, and 5 mmol/l Na-azide (pH 7.4). After 20 minutes, the reaction was stopped by the addition of 0.40 ml 20% ice-cold trichloroacetic acid. The mixture was cleared at 5000 rpm for 10 min, and to the supernatant; 3 ml of 0.1 N sodium acetate, 0.4 ml molybdate/H_2SO_4 mixture (1:1), and 0.4 ml 1% ascorbic acid were added. After 20 min, the absorbance was read at 800 nm. Ouabain at 10^{-3} M (inhibitor of Na^+/K^+-ATPase) was used in a duplicate set of each incubation to estimate the Mg^{2+}-ATPase activity. The difference in the amounts of inorganic phosphates between the two incubations was defined as the Na^+/K^+-ATPase activity and expressed as nmol Pi/mg protein/min.

Measurement of serum creatinine and blood urea nitrogen

Creatinine in the serum was determined using the Creatinine Assay Kit by Abcam® (ab65340) according to the manufacturer's protocol. Creatinine is expressed as mg/dl.

The level of blood urea nitrogen (BUN) was determined using a Urea Nitrogen (BUN) Colorimetric Detection Kit by ThermoFisher Scientific®. The assay was carried out according to the manufacturer's instructions, and BUN is expressed as mg/dl.

Measurement of serum HCO_3, Na^+ and K^+ levels

The levels of bicarbonate, sodium and potassium ions were evaluated in the serum samples using commercial kits by Randox®, according to the manufacturer's protocol.

Assessment of renal inflammation (tumour necrosis factor-alpha -TNF-α) level

The level of renal cytokine TNF-α (a marker of inflammation) was estimated in kidney homogenage samples using a mouse TNF-α ELISA kit (Abcam, UK). The assay is a solid-phase immunosorbent assay (ELISA) sandwich-enzyme-linked protocol using a 96 well micro-plate and was read at 450 nm. The concentration of TNF-α was calculated from a standard curve and expressed as pg/mL.

Estimation of renal nitrite levels

Nitrite (an indicator of nitric oxide production) was assayed by the method of Greene according to (Kuhad and Chopra, 2008). The assay mixture containing equal volumes of the kidney homogenate sample and Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was incubated for 10 min in the dark at room temperature. Afterwards, the absorbance was read at 540 nm. Nitrite concentration was obtained from a sodium nitrite standard plot and expressed as µmol/g

Estimation of renal Lipid peroxidation (MDA) level

Malondialdehyde (MDA) level is a lipid peroxidation biomarker that indicates free radical tissue damage. Lipid peroxidation was determined according to the method of Ohkawa (Ohkawa, *et al.*, 1979). This method involves the reaction of MDA with thiobarbituric acid (TBA), heated under acidic conditions to form a stable pink/reddish product (termed thiobarbituric acid reacting substances) which absorbs maximally at 532 nm. Lipid peroxidation was expressed as µmol of malondialdehyde (MDA) per mg protein.

Activity of renal anti-oxidant enzymes

Estimation of Catalase (CAT) activity in the kidney homogenate

Catalase activity was measured by a colourimetric assay method described by Hadwan (Hadwan, 2018). Assay mixture contains 0.5ml of the kidney homogenate, 1ml 10 mM hydrogen peroxide (prepared by adding 0.1134 ml of 30% hydrogen peroxide to 100ml phosphate buffer), and 1ml working solution (100 ml cobalt (II) solution, 100 ml Graham salt solution, and 1800 ml sodium bicarbonate solution). The mixture was vortexed for 5 s and kept in the dark for 10 min. The method depends on the oxidation of cobalt (II) to cobalt (III) by hydrogen peroxide in the presence of bicarbonate solution to produce a carbonate-cobaltate (III) complex ([Co $(CO_3)_3$] Co), which has an intense olive green colour. Catalase activity is directly

proportional to the dissociation rate of hydrogen peroxide in the samples, and the decrease in colour intensity represents the increase in catalase activity measured at 440 nm against the reagent blank. One unit of Catalase activity is the amount that dissociate I mM H_2O_2 in 1 min and it is expressed as U/mg protein.

Estimation of Superoxide Dismutase (SOD) activity in the kidney homogenate:

The determination of superoxide dismutase activity was conducted according to (Misra and Fridovich, 1972). The reaction involves the inhibition of epinephrine oxidation by superoxide dismutase. Briefly, 0.2 ml of the kidney homogenate was mixed with 2.5 ml of 0.05 M carbonate buffer (pH 10.2) and 0.3 ml of 0.3 mM epinephrine (freshly prepared). The increase in absorbance of the reaction mixture is monitored at 480 nm over a time course against a reference blank. One unit of SOD activity is defined as the amount of enzyme needed to cause 50% inhibition of adrenaline oxidation during one minute, and it is expressed as mmol/mg protein.

Estimation of Glutathione Peroxidase (GPx) activity in the kidney homogenate

According to the manufacturer's instructions, glutathione peroxidase (GPx) activity was measured using a commercial assay kit (Abcam, UK). The assay is a coupled reaction involving the oxidation of glutathione (GSH) to GSSG as GPx reduces cumene hydroperoxide. Glutathione reductase (GR) then reduces the GSSG back to GSH, using NADPH. The decrease of NADPH is measured at 340 nm; this is proportional to GPx activity and is expressed as pg/ml.

Protein Assay of the kidney homogenate

Tissue protein concentration was estimated as previously described by Bradford (Bradford, 1976) using bovine serum albumin as the standard and expressed as µg protein/ml

Data analysis and statistics

Results were expressed as mean ± standard error (SEM). Statistical analysis and comparison between groups were performed by analysis of variance (ANOVA) and Tukey's multiple comparison tests using the Graphpad Prism 9 software. Value of $p<0.05$ was regarded as the level of significance.

RESULTS AND DISCUSSION

Result

The result of the relative kidney weight of the experimental rats is in figure 1. The result shows a significant ($p<0.001$) increase in the relative kidney weight of the DM-untreated group compared to the control group. However, the DM-treated 1 & 2 groups had similar relative kidney weight as the control group.

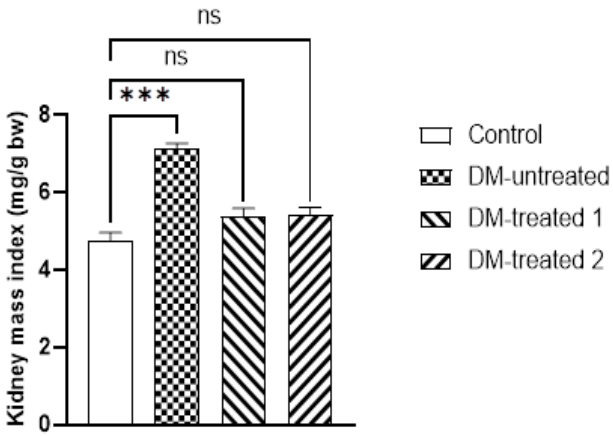

Figure 1. Impact of Cellgevity® on The Relative Kidney Weight of Diabetic Rats.

The data are expressed as mean, with the error bar showing SEM. *** represents the value of $P < 0.001$ and ns means "not significant". Comparisons are between the control group and each of the other groups, respectively.

Figure 2 shows the result of the activity of renal Na^+/K^+-ATPase across the experimental groups. There was a significant ($p<0.001$) decrease in the Na^+/K^+-ATPase activity for the DM-untreated group against the control group, while the DM-treated 1 & 2 groups showed substantial recovery in their renal Na^+/K^+-ATPase activities compared to the DM-untreated group. Significantly, there was the complete restoration of Na^+/K^+-ATPase activity for the DM-treated 2 group, similar to the control group.

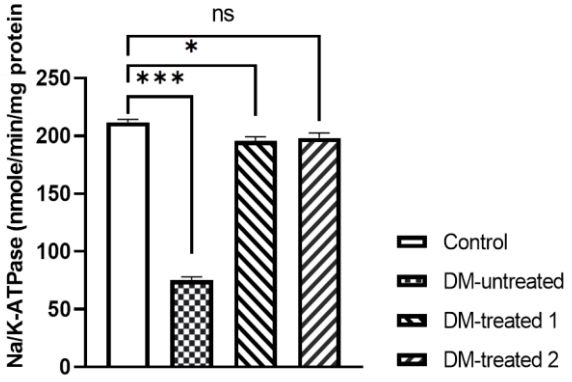

Figure 2. Impact of Cellgevity® on The Renal Na^+/K^+-ATPase Activity of Diabetic Rats.

The data are expressed as mean, with the error bar showing SEM. *** represents the value of $P < 0.001$, and * represents the value of $P < 0.05$ Comparisons are between the control group and each of the other groups, respectively.

The impairments of kidney function were determined by assessing the levels of serum creatinine, blood urea nitrogen (BUN) and serum electrolytes (HCO_3, Na^+ and K^+). The result in Figures 3A and 3B shows significant ($P < 0.001$) increases in serum creatinine and BUN concentrations in DM-untreated rats against the control group. The Cellgevity treated diabetic groups (DM-treated 1&2) have significantly decreased values compared to the untreated diabetic group. The DM-treated 2 group shows similar values compared to the control group.

Figures 3C and 3E show that there were significant ($P < 0.001$) increases in the serum levels of potassium (3C) and bicarbonate (3E) ions while there was significant decrease ($P < 0.001$) in serum sodium ion level (Fig 3D) of the untreated diabetic rats compared to the control. However, the Cellgevity® treated diabetic rats show significant improvement in their serum ion levels compared to the untreated diabetic group.

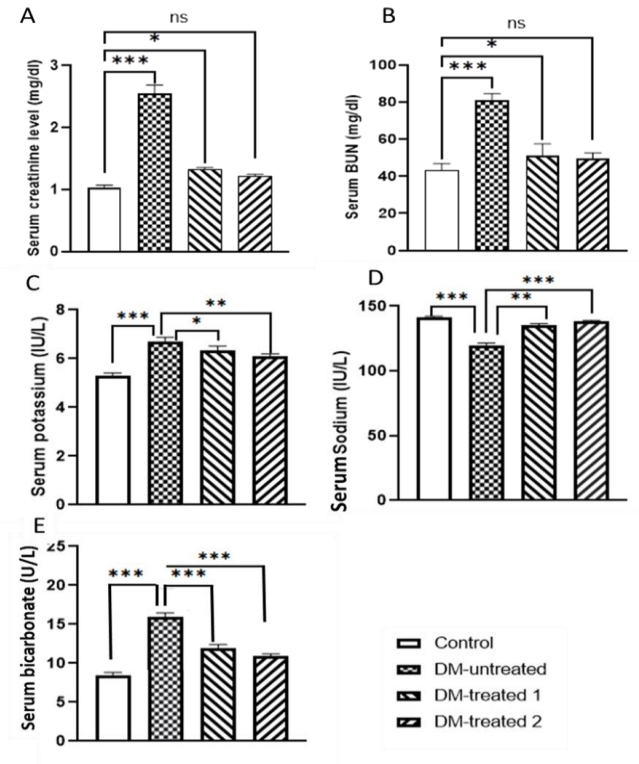

Figure 3. Impact of Cellgevity® on Biomarkers of Kidney Function of Diabetic Rats.

The data are expressed as mean, with the error bar showing SEM. *** represents the value of $P < 0.001$, ** represents a value of $P < 0.01$ and * represents the value of $P < 0.05$. Comparisons are between the control group and each of the other groups, respectively, and between the Cellgevity® treated DM-treated groups and the DM-untreated group.

Inflammation plays a vital role in the progression and pathogenesis of diabetes-induced organ complications (Lim and Tesch, 2012). Figure 4 shows the level of kidney TNF-α across the four groups. The result shows a significant ($P < 0.001$) increase in the renal TNF-α of the DM-untreated rats against the control group. The DM-treated 1 and 2 groups show substantial decreases in their values compared to the DM-untreated. The DM-treated 2 group has a similar renal TNF-α level as the control group. The above results indicate that Cellgevity® treatment decreased the levels of renal TNF-α in the kidney of diabetic rats.

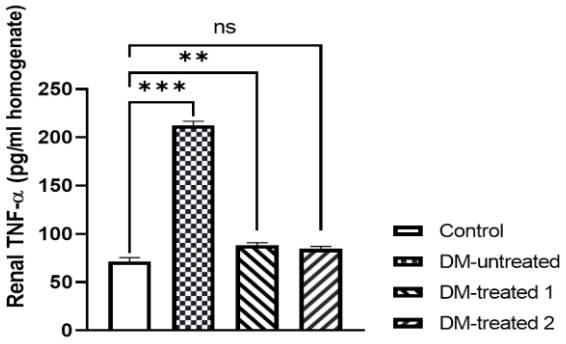

Figure 4. Impact of Cellgevity® on Kidney TNF-α. of Diabetic Rats.

The data are expressed as mean, with the error bar showing SEM. *** represents the value of $P < 0.001$ and ** represents value of $P < 0.01$. Comparisons are between the control group and the DM-untreated, DM-treated 1 and DM-treated 2 groups, respectively.

Kidney nitric oxide and nitric oxide synthase are markers of kidney endothelial function, and Figure 5 shows the effect of Cellgevity® on the level of nitrite (a marker for nitric oxide) in the kidneys of the experimental rats across the four groups. The nitrite level in the untreated diabetic group was significantly ($p < 0.0001$) increased compared with the control group. However, Cellgevity® treatment (DM-treated 1 and DM-treated 2) significantly decreased the nitrite levels

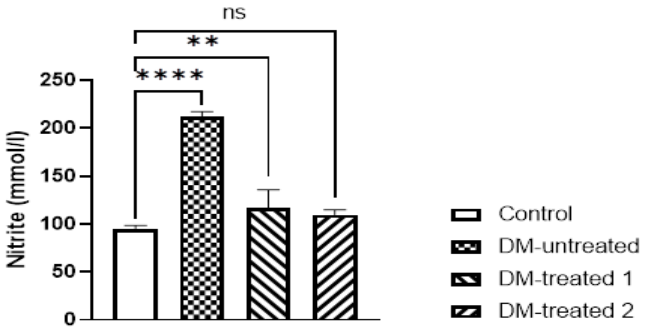

Figure 5. Impact of Cellgevity® on Kidney Nitric Oxide (nitrite) Level of Diabetic Rats.

The data are expressed as mean, with the error bar showing SEM. *** represents value of $P < 0.001$ and ** represents value of $P < 0.01$. Comparisons are between the control group and the other experimental groups, respectively.

Lipid peroxidation was elevated (MDA concentration) significantly ($p < 0.001$) in the kidney of diabetic rats compared to the control rats (Figure 6). However, treatment with Cellgevity® in the DM-treated rats significantly ($p < 0.001$) reduced the level of lipid peroxidation (MDA) as compared with the untreated diabetic group.

Figures 7A, 7B, and 7C show the activities of catalase (CAT), superoxide dismutase (SOD), and Glutathione peroxidase (GPx), respectively, across the groups. The results show that the enzymes' activities were significantly ($p < 0.001$) decreased in the DM-untreated group compared to the control group. There was a significant recovery of the enzyme's activities for the DM-treated 1 & 2 groups compared to the DM-untreated group. Significantly, the DM-treated 2 group had similar enzyme's activity profiles compared to the control group.

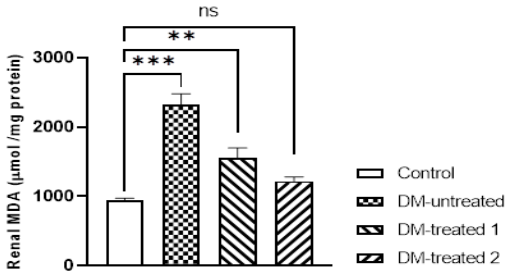

Figure 6. Impact of Cellgevity® on Kidney Lipid Peroxidation of Diabetic Rats.

The data are expressed as mean, with the error bar showing SEM. *** represents value of $P < 0.001$ and ** represents value of $P < 0.01$. Comparisons are between the control group and each of the other groups, respectively.

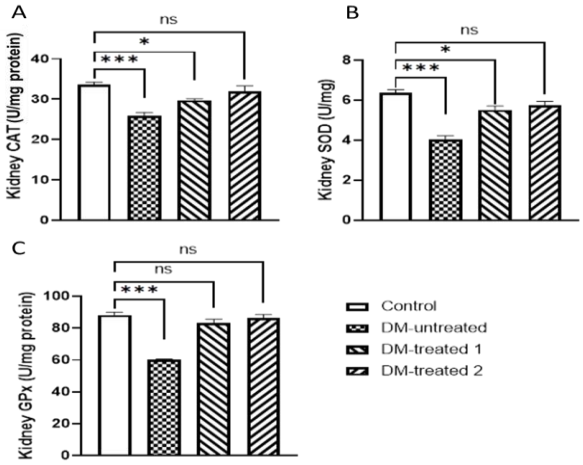

Figure 7. Impact of Cellgevity® on the Activity of Renal Anti-oxidant Enzymes of Diabetic Rats.

The data are expressed as mean, with the error bar showing SEM. *** represents a value of $P < 0.001$ and * represents $P < 0.05$ Comparisons were between the control group and the other experimental groups.

Discussion

The use of anti-oxidant based interventions has shown positive impact against diabetic kidney injuries. The overexpression of kidney superoxide dismutase (SOD) (Fujita *et al.*, 2009), treatments with lipoic acid (potent superoxide scavenger), with selenium (an integral component of glutathione peroxidases' catalytic sites), with folic acid, vitamins C and E, and with resveratrol (Rotruck *et al.*, 1973; Kuhad and Chopra, 2007; Palsamy and Subramanian, 2011; Ebaid *et al.*, 2020) have been reported. These reports highlight the potency of anti-oxidant adjuvant therapies in managing diabetic kidney complications (Lash, 2015). This study investigated the therapeutic potentials of a poly-antioxidant supplement (Cellgevity®) against STZ-induced diabetic renal injuries. The results show that untreated diabetes increased renal TNF-α, nitrite and lipid peroxidation, it decreased renal anti-oxidant enzymes activity and it altered the levels of serum blood urea nitrogen, creatinine, HCO_3, Na^+ and K^+ compared to the non-diabetic control rats.

However, the treatment of the diabetic rats with Cellgevity® at the test doses ameliorated the effect of STZ-diabetes. Significantly, the DM-treated 2 group (40 mg Cellgevity®/kg bw) had the most impact at relieving all the symptoms of kidney dysfunction monitored.

CONCLUSION

Oxidative stress is the biggest precipitator of the events that promote diabetes-induced complications. Our present results show that STZ-diabetic rats displayed symptoms of renal damage, kidney hypertrophy, impaired kidney function, renal oxidative-nitrosative stress, increased inflammatory activities and a decrease in N^+/K^+-ATPase activity. However, treatment with Cellgevity® (25 mg/kg and 40 mg/kg respectively) restored kidney function integrity dose-dependently. These results show that Cellgevity® has potent anti-oxidant and anti-inflammatory effects on STZ-diabetes kidney dysfunction, indicating that the modulation of oxidative distress and inflammation events are necessary prophylactic adjuvant steps in diabetes management. Moreover, targeting key molecules in these pathways could become a potent drug therapy intervention in the treatment of the malady

AUTHORS' CONTRIBUTIONS

OOO: Conceptualization, Methodology, Supervision, Investigation, Formal analysis, Writing- Original draft preparation, Writing- Reviewing and Editing. BOA: Methodology, Project administration, Investigation, Formal analysis, Writing- Reviewing and Editing. ENE: Methodology, Investigation, Writing- Reviewing and Editing. AAA: Investigation, Writing- Editing and Revision

ETHICS APPROVAL

Ethics approval and consent to conduct the study were approved by the Animal and Human Health Ethics Committee of Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

FUNDING STATEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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