LASU Journal of Health Sciences
www.lasucom.edu.ng; e-mail: lasu.jhs@lasucom.edu.ng
Volume 3 Issue 1(2020), pages 24 - 31

Treatment with Cellgevity® improves glycemic index and prevents atherogenic dyslipidemia in Type 2 Diabetic rat model

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ABSTRACT

Background: Type 2 Diabetic (T2DM) dependent high rate of coronary failure is attributed to the prevalence of dyslipidemia and other risk factors. The impairment of lipid metabolism in T2DM is linked to chronic oxidative stress; therefore, a rational therapeutic approach in diabetogenic management could involve the use of antioxidants. The current study evaluates the effect of Cellgevity® (a marketed glutathione supplement) on body weight, fasting blood glucose and plasma lipoprotein levels of T2DM male rats. T2DM was achieved via sucrose feeding (60%W/V) *ad libitum* for 3 weeks and streptozotocin (STZ at 55 mg/kg) intraperitoneal administration on day 22.

Method: Seventy-two hours after STZ injection, twenty one diabetic rats were divided into three groups of 7 rats each. Control and diabetic untreated (DM-untreated) groups received distilled water, diabetic treated 1 group (DM-treated 1) received Cellgevity (25mg/kg) and Diabetic treated 2 (DM-treated 2) group received Cellgevity (40 mg/kg) for twenty one days respectively.

Results: Our results show that Cellgevity significantly ($p<0.001$) reduced FBG level (40% and 45%) in DM-treated 1 & 2 respectively compared to DM-untreated animals. Serum lipoproteins were significantly distorted in the DM-untreated group compared with the Control group, however, treatment with Cellgevity® in DM-treated 1&2 significantly decrease TG (%), TC (%) and LDL (%) while HDL was significantly increased (%) compared to the DM-untreated group

Conclusion: Overall, present results show anti-hyperglycemic and anti-dyslipidemic potentials, of Cellgevity in T2DM rats; which might be via oxidative stress modulation.

Keyword: Type 2 diabetes mellitus, Cellgevity, Cardiovascular disease, Dyslipidemia, Oxidative stress

INTRODUCTION

Diabetes mellitus (DM) has become a pandemic health problem, affecting nearly 10% of the world's population[1,2]. It is a complex multifactorial disease that is influenced by individual genetic susceptibility and environmental factors, such as lifestyle[1,3-5]. DM is amplified across race, socioeconomic arrangements and age brackets[6]. Type II Diabetes mellitus (T2DM) is a complex metabolic disorder associated with impaired metabolism of carbohydrate, lipid and protein; it is characterised by insulin resistance, beta-cell impairment and hyperglycemia [4]. Chronic exposure to hyperglycemia leads to the failure of many organs causing long-term complications which results in the huge healthcare burden, morbidity and mortality associated with T2DM[3,7]. Hyperglycemia induced cellular failures in T2DM could also lead to long-term micro- and macro-vascular complications such as retinopathy, nephropathy, neuropathy, hypertension, myocardial infarction, and stroke[1,4,8,9]. T2DM share several common pathophysiological features with cardiovascular disease (CVD)[1,9-12]; these includes insulin resistance, oxidative stress, inflammation, high blood pressure, obesity, dyslipidemia and hypercoagulability[7,14,15] and report by the American Diabetes Association, indicates that cardiovascular disease (CVD) accounts for about 75-80% of mortality in T2DM[8]. Oxidative stress is known to contributes to the pathogenesis of insulin resistance and T2DM[7,16] and this could further become amplified by hyperglycemia, insulin resistance and dyslipidemia, thus leading to inflammation and endothelial dysfunction thereby promoting atherogenesis and vascular complications[3,17-19]. Current evidences also suggests that the interaction of T2DM and associated cardiovascular impairment may lead to atherosclerosis [18].

Hyperglycemia

Hyperglycemia is the hallmark of diabetes and prolonged exposure to hyperglycemia is the cause of most diabetes related complications[1,3,20]. The mechanisms of chronic hyperglycemia-induced complications include; activation of polyol pathway, protein glucation and formation of activated glycation endproducts (AGEs); leading to elevated oxidative

stress[20-23]. The polyol pathway consists of two enzymes; aldose reductase (AR) reduces glucose to sorbitol using NADPH as co-factor, while the second enzyme, sorbitol dehydrogenase (SDH) converts sorbitol to fructose utilises NAD^+ as co-factor[21]. Increased flux of glucose through the polyol pathway results in depletion of cellular NADPH and NAD[(21]; the decrease in NADPH level could result in the decreased in reduced glutathione (GSH) level while a decrease in NAD level would lead to high cellular NADH/NAD ratio thereby stimulating high NADH oxidase activity leading to high ROS production[21,24]. Elevated intracellular ROS generation can inhibit Ca^{2+}-ATPase activity amongst several effects. ROS interferes with the enzyme's ATP binding site through oxidation of its cysteine thiol residues[24] thereby inhibiting the enzymes capacity to hydrolyse ATP[24]; this could lead to contractile and endothelial dysfunctions which are implicated in pathogenesis of CVD[8,24]. Long-term accumulation of glycated biomolecules and advanced glycation end products (AGEs) is also a precursor of hyperglycemia-induced tissue damage in diabetes[20,25,26]. AGEs represent a heterogeneous group of chemical products resulting from a non-enzymatic sugar (reducing) fixation with proteins, lipids, nucleic acids, or a combination of these[20]. AGEs-protein interactions may cause oxidative stress, pro-inflammatory signalling, endothelium dysfunction, arterial stiffening, and microvascular complications[25,26].

Cardiovascular disease

Cardiovascular disease (CVD) is the umbrella name for all types of disorders that affect the heart and blood vessel[8,11,27], it includes coronary heart disease (heart attack), stroke, hypertension, peripheral vascular disease, heart failure, arrhythmias and heart valve problems[3,8,11,28]. Studies have shown that the rate of diabetes induced CVD is over three-times greater than without diabetes[3,11,27] and CVD is the most prevalent and detrimental complication of diabetes mellitus[3,11,27]. The high rate of coronary failure in diabetes is attributed to the prevalence of CVD major risk factors, such as insulin resistance, hypertension, obesity, and dyslipidemia in diabetes[7,12,29,30]. T2DM and CVD are both complex diseases having common risk factors which suggests the existence of some shared genetic locus[7,31]. The mechanisms that link cardiovascular risk in diabetes are complex, multifactorial and poorly understood; however, associations between chronic hyperglycemia and intracellular metabolic changes resulting in oxidative stress, low-grade inflammation, and endothelial dysfunction have been suggested as a putative factor linking the two syndromes[27]. This is clearly not the only factor, since both pre-diabetes and the presence of the metabolic syndrome, even in euglycemic patients; increase the risk of most types of CVD[27]. The link between diabetes and CVD defects also exist in vascular dysfunction and the molecular mechanisms involved in insulin-resistance and hyperglycemia which are well reviewed in[27].

Insulin Resistance

Insulin resistance (IR) occurs when the nutrient energy storage pathways (evolved to manage energy utilization) are exposed to chronic caloric surplus[32]. IR is classically defined as a decreased sensitivity of respondent tissues to normal circulating levels of insulin (32). Insulin is a major regulatory anabolic hormone, responsible for maintenance of glucose homeostasis and its primary targets include the skeletal and cardiac muscles, adipose tissue and liver[32,33]. Insulin is secreted by the pancreatic β cells in response to elevated postprandial levels of circulating glucose and amino acids and it acts by; increasing the rate of glucose uptake (primarily into skeletal muscle and adipose tissue), reducing hepatic glucose production (by decreasing gluconeogenesis and glycogenolysis), increasing lipid synthesis in liver and fat cells, and decreasing fatty acid release from adipose tissue[32,34]. Insulin resistance is characterized by a decline in cellular response to insulin stimulation by these tissues and it plays a major role in the pathogenesis of type 2 diabetes mellitus and it is implicated in some other clinical disorders, such as obesity, dyslipidaemia and hypertension clustering in the so-called metabolic syndrome[33,35]. Dysregulated fatty acid metabolism and ectopic lipid accumulation play important roles in the induction of insulin resistance in skeletal muscle (the major site of glucose uptake). The mechanisms involved in the pathophysiology of insulin resistance is multifactorial involving multiple defects in insulin signal transduction, impaired glucose metabolism and disposal, ectopic fat accumulation, pro-inflammatory signalling and lipid metabolism dysregulation; these are eminently review elsewhere[32,3]

Dyslipidemia

Dyslipidemia characterised by a lipid triad comprising; high plasma triglyceride concentration (TG), low HDL cholesterol concentration and elevated concentration of small dense LDL-cholesterol particles is a major risk factors for cardiovascular disease in diabetes mellitus[11,35-38]. Other risk factors (obesity, insulin resistance hypertension), collectively referred to as metabolic syndrome (MetS)[31,39] also increase predisposition of diabetes to cardiovascular disease[6,35,40-42]. Atherogenic dyslipidemia in T2DM is particularly characterised by elevated serum concentrations of TG-rich lipoproteins (TRLs)[43] and the core lipoprotein abnormality is an increase in large TG-rich very-low-density lipoprotein (VLDL)[7,43]. Lipid derangement in T2DM may be caused by several factors such as insulin resistance, hyperglycemia, adipocytokines and oxidative stress[44,45]. Particularly, insulin resistance enhances release of non-esterified fatty acids (NEFA) from triglycerides stored in adipose tissues thereby causing increased synthesis and reduced clearance of hepatic triglyceride[32,43,46] which leads to hepatic synthesis of triglyceride-laden

very low density lipoprotein cholesterol (VLDL-C) and increased secretion of ApoB[7,45]. Triglyceride-laden VLDL enrichs LDL and HDL making them rich in cholesterol and the triglyceride-rich LDL molecules are subsequently hydrolyzed to form highly atherogenic small dense LDL [47-50] that is a notorious factor for microvascular dysfunctions and cardiovascular impairments [11,46]. Insulin resistance may also lead to downregulation of lipoprotein lipase (responsible for clearance of circulating triglyceride) thereby contributing to postprandial lipemia and ectopic lipid accumulation[37]. The pathophysiology of dyslipidemia in T2DM is a rather complex metabolic event involving insulin resistance, chronic oxidative stress and hyperglycemia[35,42,43,48].

Oxidative stress is an important factor implicated in the development and progression of type 2 diabetes mellitus (T2DM) and its complications. Chronic oxidative stress causes several cellular and metabolic dysfunctions such as insulin resistance, dyslipidemia, β-cell impairment and glucose resistance[35]. Oxidation of cell membranes and circulating lipoprotein lipids is a major risk factor for the development of vascular disease in diabetes; free radicals and reactive oxygen species (ROS) interact with lipid bilayer of cell membrane resulting in lipid peroxidation, cell membrane leakiness, and inactivation of membrane bound proteins (enzymes, receptors and pumps) leading to compromise of cellular processes. Small and dense LDL (sdLDL) is easily oxidized and high levels of this oxidised-sdLDL are associated with foam cell formation and atherosclerosis which is tightly linked to the pathogenesis of cardiovascular disease. A rational approach in diabetogenic management may involve antioxidant therapy. Such approach may shed light into the therapeutic role of antioxidants in stalling and or ameliorating the effect of oxidative stress in diabetogenesis.

MATERIALS AND METHODS

Drugs and chemicals

Cellgevity® by Max international, USA was purchased from an accredited distributor in Sagamu, Nigeria. Streptozotocin (STZ) was purchased from Sigma Aldrich and table sugar (sucrose) was obtained at the local market. Total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) assay kits were obtained from Randox Laboratory (Crumlin, UK). Other chemicals and reagents used were of analytical grade

Animals and Experimental design

The study was conducted in compliance with established protocol of biomedical research for the use of experimental animals as approved by the Animal and Human Health Ethics Committee of Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye, Nigeria. The study was carried out in the Department of Biochemistry, Faculty of basic Medical Science, Olabisi Onabanjo University, Nigeria. Twenty eight (28) healthy adult male Wistar rats weighing 210g ± 30g were maintained in well-ventilated cages at normal room temperature (28°C ± 3°C) with a 12 hour natural light–dark cycle and provided fed and water *ad libitum*.

Animal feed

The rats were feed with normal rat chow during the acclimatization period. After acclimatization, animals were feed according to their groups. The control group received normal rat chow while others groups (DM-untreated, DM-treated 1 and DM-treated 2) were maintained on a high-sucrose diet (comprising of 60% sucrose) [51] throughout the study.

Induction of Type 2 diabetes

T2DM was achieved by sucrose feeding (20%W/V) ad libitum for 3 weeks and streptozotocin (55 mg/kg i.p.) on day 22. Seventy-two hours after STZ injection, twenty one (21) diabetic rats (showing hyperglycemia) were divided into three groups of 7 rats each: DM-untreated, DM-treated 1 (Cellgevity 25 mg/kg), and DM-treated 2 (Cellgevity 40 mg/kg). Treatments were administered as single oral daily doses. Control and DM-untreated groups received 2ml distilled water.

Necropsy

At the end of the dosing period, rats were sacrificed by cervical dislocation 24 hr after the last treatment, and blood was collected by cardiac puncture into plain pre-chilled bottles on ice and allowed to stand for 1 hour. Serum was collected into fresh sample bottles after blood was centrifuged at 3000 rpm for 15 mins and stored at -20°C for subsequent biochemical analyses.

Assays

Measurement of Fasting blood glucose

The animal blood glucose was measured weekly after a 12 hr fasting period using AccuChek Active© glucometer and glucose strips with blood obtained by tail vein puncture. Measurements were done in triplicates.

Assessment of plasma lipoprotein parameters

Commercial kits by Randox Laboratories Ltd. (Crumlin, UK) were used to measure serum total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL) levels; these were estimated following the cholesterol oxidase, glycerol-3- phosphate oxidase and HDL Cholesterol-Direct Clearance methods respectively according to the manufacturers' instructions. The Freidewald formula was used to extrapolate serum low-density lipoprotein (LDL) [52].

Lipoprotein ratios:

The following formulas were used for lipipoprotein ratios

Atherogenic index (AI) was calculated by:

$$AI = TG/HDL$$

Cardiovascular risk ratio (CRR) was calculated by:

$$CRR = TC/HDL$$

Atherogenic coefficient (AC) was calculated by:

$$AC = (TC - HDL)/HDL$$

Atherogenic index of plasma (AIP) :

$$AIP = \log(TG/HDL)$$

Lipoprotein ratios are defined to optimize the predictive capacity of the serum lipid profile and can be used as markers of insulin resistance, atherosclerosis and CVD risk factor. They represent indicators having superior predictive value than independent lipid parameters[(53,54].

Statistical analysis

All data are expressed as mean ± SEM. Statistical analyses between experimental groups and the control group were carried out using one-way ANOVA followed by tukey's multiple comparison tests. Values for $p<0.05$ are considered to be statistically significant. Graphpad Prism 8® software was employed for these analyses.

RESULTS

The effects of Cellgevity on fasting blood glucose (FBG) levels

Figure 1 shows effects of Cellgevity on fasting blood glucose (FBG) levels in normal and diabetic rats. Prior to STZ treatment, the FBG level across all the groups did not show any significant difference (data not shown). However, following the induction of diabetes, the result in fig 1 shows that FBG of DM-untreated group increased significantly ($p < 0.001$) by 130% when compared with control group. DM-treated 1 & 2 groups show significant ($p < 0.01$ and $p<0.001$) decreases in FBG by 40% and 45% respectively when compared with DM-untreated group. FBG in DM-treated 1&2 groups were slightly higher than control.

The effects of Cellgevity on body weight

The result showing effect of Cellgevity on body weight in normal and diabetic rats is given in fig 2. The result represents the final mean body weight of the groups. The result shows no significant differences in the animals' mean body weight across the four groups.

The effects of Cellgevity on lipid parameters

Results showing effect of Cellgevity on lipid parameters is given in figure 3. The results show that DM-untreated rats have increased ($p < 0.05$) serum total cholesterol (37%), triglycerides (76%), and low density lipoprotein (160%) when compared with the control group. The HDL level in DM-untreated group was reduced by 35% ($p > 0.05$) compared with control normal. DM-treated 1&2 groups show a reversal of dyslipidemia compared to DM-untreated; there were significant ($p<0.001$) decreases in TC, TG and LDL , there were also significant increases in the HDL levels compared to the DM-untreated group.

Lipid ratios

The results representing lipoprotein ratios: atherogenic index, cardiovascular risk index, atherogenic index of plasma and atherogenic coefficient are presented in fig 4. DM-untreated group has significant ($p<0.001$) increases in atherogenic index, cardiovascular risk index, atherogenic index of plasma and atherogenic coefficient compared to the control group, while the DM-treated 1&2 groups show comparable values for the indices compared with the control group.

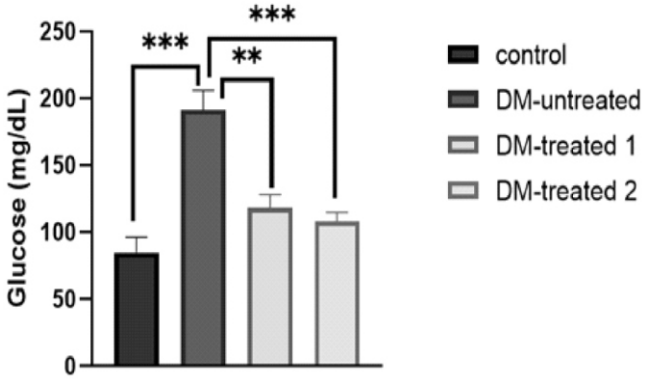

Figure 1: Fasting blood glucose (FBG) levels in normal and diabetic rats

Result is expressed as mean ± SEM. Statistical analysis was carried out using one way ANOVA followed by Tukey's multiple comparison tests. Two and three asterisks indicates significance at $p<0.01$ and $p<0.001$ respectively

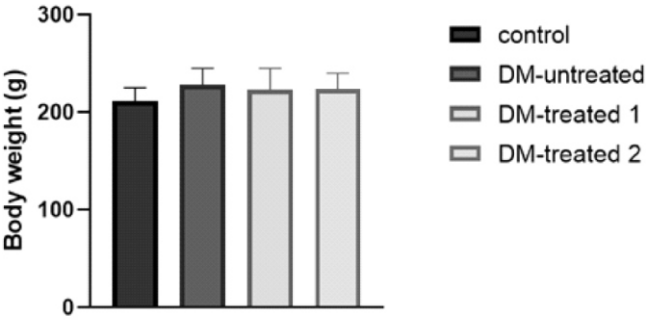

Figure 2: Chart showing the final mean body weight of control and diabetic groups

Result is expressed as mean ± SEM. Statistical analysis was carried out using one way ANOVA followed by Tukey's multiple comparison tests.

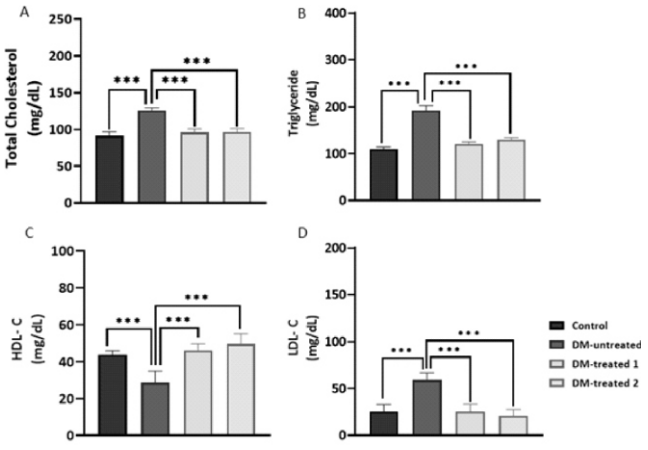

Figure 3: Effects of Cellgevity on lipid parameters in control and diabetic male rats. Bar charts represent the mean serum Triglyceride (A), Total cholesterol (B), High density lipoprotein cholesterol HDL (C) and Low density lipoprotein cholesterol LDL (D) for control and diabetic groups. Result is expressed as mean ± SEM. Graphpad Prism® 8 software was used for the statistical analysis; one way ANOVA analysis followed by Tukey's multiple comparison tests. Three asterisks indicate significance of p<0.001

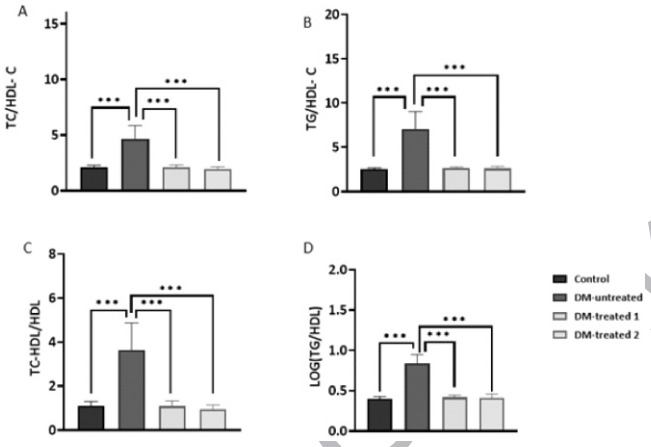

Figure 4: Effect of Cellgevity on lipoprotein ratios in the control and diabetic male rats. Figures 4A, 4B, 4C and 4D represent the results of cardiovascular risk ratio, cardiovascular risk ratio, atherogenic coefficient and atherogenic index of plasma respectively for control and diabetic male rats. Result is expressed as mean ± SEM. Graphpad Prism®8 software was used for the statistical analysis; one way ANOVA analysis followed by Tukey's multiple comparison tests. Three asterisks indicate significance of p<0.001

DISCUSSION

T2DM has become a health pandemic, affecting about 8% of the world's population and becoming increasingly popular in low and middle income countries[55]. Due to the increasing prevalence, there is a growing need to develop integrated approaches toward diabetogenic management and prevention by exploring the potentials offered by less invasive therapies such as antioxidant supplementation amongst other therapies. Dyslipidemia characterised by increases in TC, TG, and LDL and decrease in HDL is a nagging factor for the development of CVDs in T2DM[56] and atherogenic dyslipidemia characterised by high LDL/HDL ratio and high TAG level, is high predictor of cardiovascular risk [42,43]. Risk of carotid atherosclerosis and stiffness has equally been linked to high levels of serum TC and TG with low-serum HDL levels (18). Other markers of atherogenic dyslipidemia includes atherogenic index (AI) and cardiovascular risk index (CRI) which are strong predictors of atherosclerosis and coronary heart disease risks (38,43,49).

Results of this study show that the TG, TC, LDL-C and lipid ratios all increased significantly (p<0.001) at 30%, 77% , 126% and >250% respectively in diabetic untreated rats compared with the control group; this result indicates elevated risk for cardiovascular dysfunction in the diabetic animals (3,47). Conversely, treatment of diabetic rats with Cellgevity show significant (p<0.001) decreases in; TG, TC, LDL-C, CRI and AI compared with DM-untreated rats. Cellgevity is marketed to contain ingredients which help support the production and functions of glutathione (57,58). Glutathione (γ-glutamyl-cysteinylglycine) is a major endogenous antioxidant which plays important roles in combating oxidant effects and oxidative stress (59,60). Curiously; T2DM has been linked with depletion of cellular glutathione, particularly in the erythrocytes [60,61] and impaired glutathione metabolism occasioned by hyperglycemia has been suggested as putative cause of glutathione depletion in T2DM[60]. Oxidative stress is significantly increased in chronic hyperglycemia due to elevated ROS production and depletion of endogenous antioxidants[6,62]. This could evidently be due to reduced rate of antioxidant/ precursor synthesis and increased irreversible antioxidant utilization leading to low glutathione concentration in T2DM[60]. Supplementation with glutathione could therefore help to combat oxidative stress in T2DM and protects against hyperlipidemia, lipid peroxidation, vascular damage and CVD. From the present results, treatment with Cellgevity stalls diabetes induced atherogenic dyslipidemia in the male rats suggesting its therapeutic capability.

Elevated blood glucose is the hallmark of T2DM and present results show significant (p<0.001) increase (129%) in FBG of DM-untreated rats compared to control rats, however, DM-treated groups (Cellgevity 25mg/kg and 40mg/kg) show signifant decreases in FBG level compared to DM-untreated. Glutathione may improve glycemic index in T2DM by reducing oxidative stress and its effects. Hyperglycemia induced oxidative stress is a primary factor in the progression of T2DM and its complications (16). Oxidative stress increases the levels of pro-inflammatory proteins such as cytokines; leadings to local and systemic inflammation which contributes to insulin resistance induced hyperglycaemia[63,64]. Results suggest that supplementation with antioxidant Cellgevity may complement cellular glutathione synthesis thereby reducing oxidative stress, insulin resistance and hyperglycemia.

Overall, supplementation with Cellgevity improves the lipid profile, lipid ratios and glycemic index in T2DM male rats compared to untreated group.

CONCLUSION

The results from this study demonstrate antihyperglycemic and antidyslipidemic potentials of Cellgevity in experimental T2DM male rats. Although specific mechanisms of actions were not determined, however, it might be, due to modulation of oxidative stress (an important factor in the pathogenesis of the disease) in the diabetic animals. Cellgevity is marketed to contain glutathione promoting supplements. Improvement in endogenous glutathione synthesis and concentration through Cellgevity supplement administration, may aid the inhibition of ROS's cellular insult and system recovery in diabetic rats. The result of this study provides incentives for further translational studies in humans; to explore the roles of antioxidants in diabetogenic management and therapy.

ACKNOWLEDGEMENT

The authors wish to acknowledge Mr Femi Museliu (laboratory technologist) at the department of Biochemistry, Olabisi Onabanjo University, Nigeria from his technical support.

Authors' contributions OOO and ABO – Research design, methodology, project administration, original draft writing, writing (review & editing). EAA, ASA, OOR - analysis, Investigation, writing (review & editing). All authors funded, and approved the manuscript.

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

Disclosure of competing interest: None

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