NSP. The level of G6PDH after cold stress exposure in rats is depicted in Figure 5b. G6PDH level was a significant higher in STP group compared with control (NSP). Cellgevity® administration in STP group elevated G6PDH level significantly ($p<0.05$) when compared with NSP and STP.

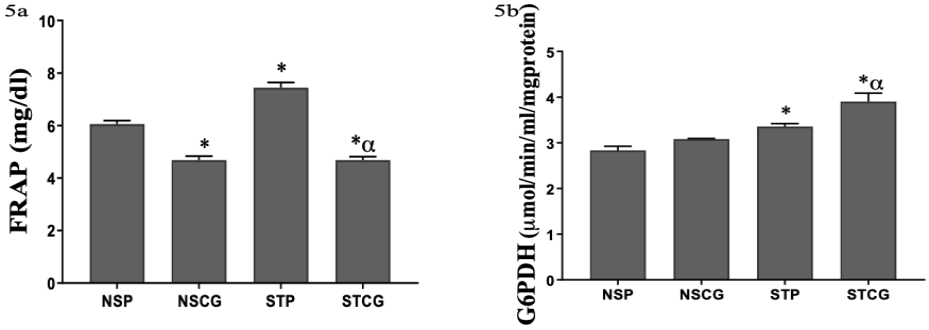

Figure 5a Effect of Cellgevity® on FRAP level in cold-restraint rats.

Figure 5b Effect of Cellgevity® on G6PDH level in cold-restraint rats. Values expressed as mean ± SEM, n= 6 per group, $p < 0.05$ *vs* EWUrmXñ *vs* STP (one-way ANOVA followed by SNK post hoc test).

Effect of Cellgevity® on Alpha Amylase and Alpha Glucosidase levels

Alpha amylase level after cold restraint stress exposure in rats is depicted in Figure 6a. The alpha amylase level is apparently decreased in cold-restraint rats (STP) compared with control rats. After administration of Cellgevity®, alpha amylase level was significantly increased ($p<0.05$) in STCG rats compared with NSP and STP rats. NSCG also had a significant increase in alpha amylase level compared with NSP.

The level of alpha glucosidase is depicted in Figure 6b. The alpha glucosidase level in STP rats is apparently lower compared with control rats (NSP). However, after administration of Cellgevity® a significant decrease ($p<0.05$) was observed in the alpha glucosidase level in STCG rats compared to NSP and STP.

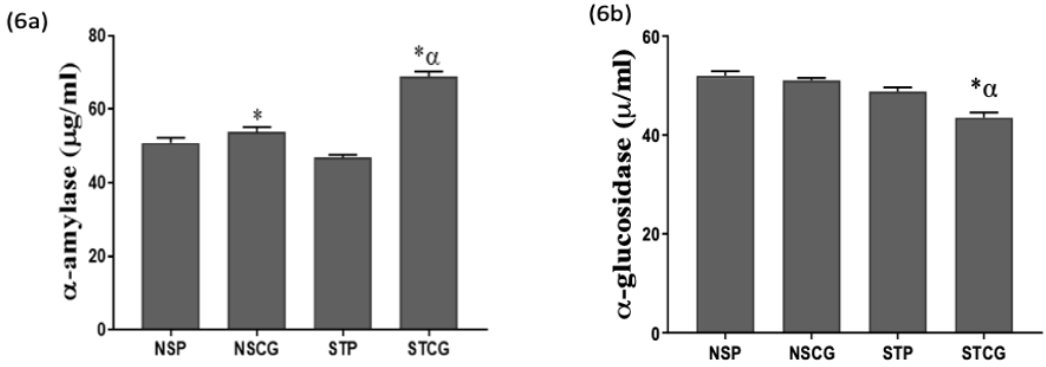

Figure 6a ((ßooP -amylase level in cold-restraint rats.

Figure 6b Effect of Cellgevity -glucosidase level in cold-restraint rats. Values expressed as mean ± SEM, n= 6 per group, p< 0.05 *vs* STP (one-way ANOVA followed by SNK post hoc test).

DISCUSSION

This present study examined the glucometabolic potential of Cellgevity® under stress conditions in male Sprague-Dawley rats. We employed cold restraint stress to effectively induce a physical and psychological stress condition [18]. Cellgevity® is a dietary supplement that

contains D-ribose and L-cysteine, two main molecules that boost intracellular GSH synthesis and concentrations, lowering oxidative stress [24]. The recent rise in antioxidant treatment further clarifies why dietary antioxidant has continually been at the forefront of preventive medicine [25-26]. Our findings reveal that cold restraint stress increases oxidative stress level and corticosterone levels as well as promotes insulin resistance. Furthermore, there was an increase in the level of insulin, but cold restraint stress did not alter glucose tolerance. However, Cellgevity® modulates some of the oxidative damage induced by cold restraint stress [27-28].

Different studies have shown that stress deteriorates glycaemic and induces metabolic dysfunction such as impaired glucose tolerance and insulin insensitivity, hyperinsulinemia, hyperglycemia, and increased corticosterone levels Results from our study indicated that Cellgevity® supplementation reduced significantly, the increased blood glucose induced by cold restraint stress. We observed an altered insulin sensitivity induced by stress, while glucose tolerance was unaffected. The oral glucose tolerance test (OGTT) is commonly used in clinical settings to assess apparent insulin resistance and insulin resistance. [29]. When insulin fails to increase glucose absorption and reduce hepatic glucose synthesis, insulin resistance is evident. Insulin is a critical regulator of carbohydrate metabolism and serves as the primary regulator of glucose levels by increasing glucose absorption into insulin-sensitive organs such as the liver and skeletal muscles [30-31]. Considering the importance of insulin in glucose metabolism, we measured the fasting plasma insulin level and observed an elevated insulin concentration in response to cold restraint stress. As a result, the resulting hyperinsulinemia and decreased glucose clearance after insulin challenge in cold restraint rats suggest impaired insulin sensitivity and glucose control. Our data further shows Cellgevity® supplementation ameliorate insulin sensitivity as well as cause a significant reduction in fasting plasma insulin levels in male Sprague-Dawley rats. This corroborates the perception that dietary antioxidants improve insulin sensitivity by eliminating to a larger extent some of the oxidative damage to insulin-responsive tissues.

The physiological responses to acute and chronic stressors have been observed to differ in rodents [32-33]. ACTH is released in response to stress, and it acts on the adrenal cortex to induce the production and release of corticosterone [34]. Corticosterone elevates blood glucose levels by mobilizing stored energy reserves in body tissues [35]. Corticosterone stimulates gluconeogenesis in the liver [36], and can also cause damage to the liver tissue by increasing oxidative stress [37]. In this study, the elevated stress level was indicative as levels of corticosterone in stressed rats significantly increased. However, our data shows that the administration of Cellgevity® modulates the effect of stress by reducing corticosterone levels. Furthermore, lower corticosterone levels improved glucose uptake, [38] allowing glycemia to be controlled even after stress induction. Recent research has linked stress-induced increases in glucocorticoids to glucose control dysregulation [39]. Stress also increases the generation of reactive oxygen species (ROS), which causes lipid peroxidationUnder stress, our bodies produce more ROS than antioxidant species, resulting in an imbalance that can damage cellular components such as lipids, protein, and DNA. Our data suggest that lipid peroxidation induced by stress is slightly modulated by Cellgevity® supplementation. Although in contrast to our hypothesis, the GSH level was comparable between stressed rats with or without Cellgevity® supplementation. Also, the total antioxidant capacity from the FRAP result in our

study shows contrasting values. While stressed animals have increased total antioxidant activity, Cellgevity® supplementation shows a reduced FRAP value. Next, we measured the effect of Cellgevity® supplementation on liver damage. The etiopathogenesis of liver diseases is widely assessed by enzyme levels such as ALT, AST, and ALP. Stress-induced liver damage has been extensively investigated [40].

Data from our current research shows that exposure of rats to cold restraint stress resulted in marked liver damage, as evidenced by a significant increase in serum enzyme concentrations (ALT, ALP, and AST). Cellgevity® supplementation however did not attenuate the effect of stress on the liver as depicted in our data. The lipid profile after rats' exposure to cold restraint stress was also evaluated in the current study. It is well known that adipose tissue regulates energy homeostasis as well as the metabolism of glucose and lipid dynamically and critically [41]. Dyslipidemia has an important role in metabolic disorders and is a significant contributor to insulin resistance. This study's investigation of the lipid profile revealed that the level of TRIG in cold-restraint rats was noticeably higher than controls. Insulin resistance has been linked to elevated TRIG levels, and in a similar way, elevated corticosterone levels have been linked to resistance to insulin [42-43]. Although our result suggests modulation of stress-induced hyperlipidemia, there was a decline in HDL level after Cellgevity® supplementation.

Activities of some enzymes involved in glucose metabolism were also determined. For instance, G6PD, which catalyzes the oxidation of glucose-6-phosphate to satisfy the cellular needs for reductive biosynthesis and the maintenance of cellular redox state, was evaluated. G6PD-deficient cells that have been damaged may impair the normal physiological activities of many tissues. In this study, we observed G6PD expression was enhanced in Cellgevity® treated rats after cold restraint stress.

Alpha-amylase is an enzyme present in saliva and pancreatic juice that catalyzes the hydrolysis of starch to smaller oligosaccharides, which are then degraded to glucose by alpha-glucosidase, an enzyme found at the mucosal brush border of the small intestine. Alpha-glucosidase and alpha-amylase inhibitors could be useful in the development of medications to treat obesity, diabetes, and hyperlipidemia [44]. Cellgevity® elicited a higher inhibition on the activity of alpha-glucosidase than alpha-amylase, which may be a positive indicator in eliminating the negative side effects associated with traditional alpha-glucosidase and alpha-amylase inhibitors.

CONCLUSION

Findings from our study suggest that cold restraint stress-linked disruption of glucometabolic indices in rats involves mechanisms leading to insulin resistance, hyperlipidemia, elevated oxidative stress, and higher corticosterone levels. Additionally, Cellgevity® supplementation showed modestly positive effect on stress-induced damage notably by reducing the serum corticosterone levels and modulating both serum oxidative stress markers and metabolic parameters. Interestingly we did not observe significant improvements in liver damage, hyperlipidemia or the GSH boosting effects of Cellgevity®. This might be attributed to the relatively short duration of our study. However, the usage of this dietary antioxidant against stress-induced damage should be taken with medical recommendation. Taken together, Cellgevity® on cold restraint rat models appeared to enhance glucometabolic functions by potentially exhibiting a mild beneficial effect.

Abbreviation: ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; AUC: Area under the curve; CAT: Catalase; CG: Cellgevity®; CHOL: Total cholesterol; DNA: Deoxyribonucleic acid; DNTB: Ellman's 5, 5 'dithiobis (2-nitrobenzoic acid); FBG: Fasting glucose level; FINS: Fasting insulin level; FRAP: Ferric ion reducing antioxidant power; G6PDH: Glucose-6-Phosphate Dehydrogenase; GSH: Reduced glutathione; HDL: High-density lipoprotein; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; IRI: Insulin resistance index; ISI: Insulin sensitivity index; LDL: Low-density lipoprotein; MDA:Malonaldehyde; NADPH: Nicotinamide adenine dinucleotide phosphate hydrogen; OGTT: Oral glucose tolerance test; pNP: p-nitro phenol; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TPTZ: 2,4,6-Tris (2-pyridyl)-s-triazine; TRIG: Triglycerides

Competing interest: The authors have no competing interest to declare.

Authors' contribution: Dr. Morakinyo A, Prof. Samuel T.A and Mr. Mofolorunso A were involved in the conceptualization, design and writing of the manuscript. Mr. Mofolorunso helped in the statistical analysis and drafting of the manuscript. Oyebanji KE and Ndubuisi C also contributed to the writing and editing of the manuscript.

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