Research Article **Open-Access**

Beneficial effect of Cellgevity® on metabolic indices, lipid profile, and antioxidant enzymes in cold-restraint stress rat model

Adekunle Muiz Mofolorunso[1]*, Kehinde Emmanuela Oyebanji[2], Chinenye Nneamaka Ndubuisi[2], Titilola Aderonke Samuel[3], Ayodele Olufemi Morakinyo[2]

[1]Department of Physiology, Babcock University School of Basic Medical Science, Ilishan, Ogun State, 121003, Nigeria.
[2]Department of Physiology, College of Medicine, University of Lagos, Idi-Araba, Lagos State, 101014, Nigeria.
[3]Department of Biochemistry, College of Medicine, University of Lagos, Idi-Araba, Lagos State, 101014, Nigeria.

***Corresponding Author:** Adekunle Muiz Mofolorunso, Department of Physiology, Babcock University School of Basic Medical Science, Ilishan, Ogun State, 121003, Nigeria

Submission Date: August 31st, 2023; **Acceptance Date**: October 3rd, 2023; **Publication Date:** October 11th, 2023

Please cite this article as: Mofolorunso A.M., Oyebanji K. E., Ndubuisi C. N., Samuel T. A., Morakinyo A. O. Beneficial effect of Cellgevity® on metabolic indices, lipid profile, and antioxidant enzymes in cold-restraint stress rat model. Dietary Supplements and Nutraceuticals 2023; 2(10): 41-54. DOI: https://www.doi.org/10.31989/dsn.v2i10.1223

ABSTRACT

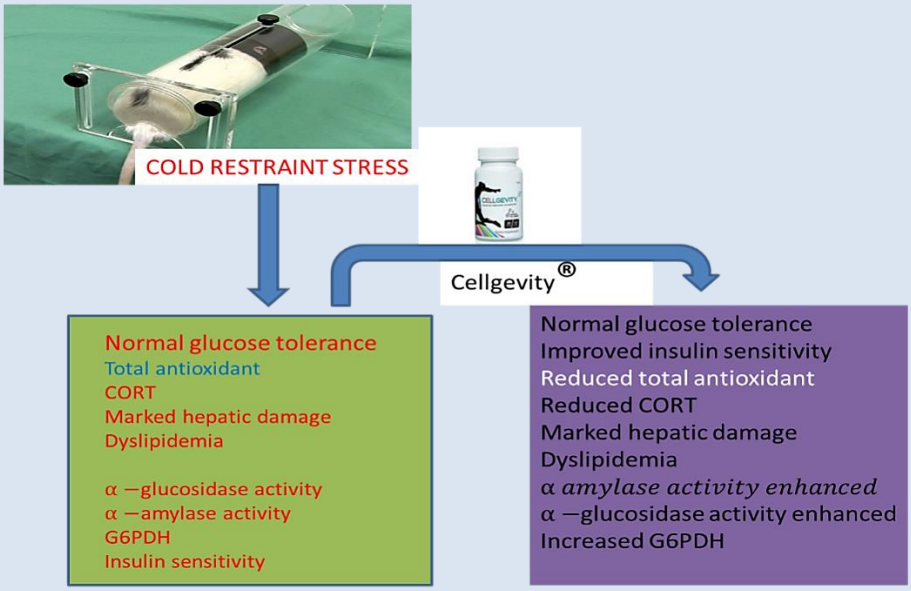
Background: Stress is a state of imbalance homeostatic environment caused by a psychological, environmental, or physiologic stressor. Stress is a common risk factor that is involved in the etiopathogenesis of diverse disorders. Moreover, stress hormones have an immediate negative impact on glycemic management.

Purpose of Study: The aim of our study is to investigate the glucometabolic potential of Cellgevity® under stress conditions in male Sprague-Dawley rats. We employed cold restraint stress to effectively induce a physically and psychologically stressed condition.

Methods: Twenty-four (24) male Sprague-Dawley rats (207±20g) were divided into four groups at random: No stress Placebo (NSP), no stress cellgevity (NSCG), stress placebo (STP) and stress cellgevity (STCG). The restraint (immobilization) stress was performed 2hr daily in the morning using a cylindrical cage (8:00 h± 10:00 h.), and the cold stress was performed for 2hr in the afternoon (16:00 h± 18:00 h). The enzyme-linked immunosorbent assay was used to assess the circulating insulin and corticosterone levels. The glucose tolerance test (GTT) and insulin tolerance test (ITT), as well as the expression of G6PDH, alpha-amylase, and alpha-glucosidase enzymes were evaluated for glucose metabolism. Biochemical parameters such as Lipid profile, liver enzymes, and oxidative stress parameters were also determined.

Results: The STP rats had increased oxidative stress levels, elevated corticosterone levels, insulin resistance, dyslipidemia, and oxidative liver damage. Furthermore, cold restraint stress improved insulin levels but had no effect on glucose tolerance. However, Cellgevity® mitigates cold restraint stress-induced oxidative damage by causing reduction in corticosterone levels, modulating serum oxidative stress markers as well as metabolic indices.

Keywords: Cold-restraint Stress, Glucose Tolerance, Insulin Sensitivity, Corticosterone, oxidative stress.



INTRODUCTION

Stress, a perturbation of homeostasis triggered by psychological, environmental, or physiological stressors [1], constitutes an adaptive biological response to disturbances that unsettle equilibrium [2]. Stress constitutes a pervasive risk factor, implicated in 75%–90% of diseases, and is intricately linked with the etiopathogenesis of diverse disorders, ranging from peptic ulcers and insulin resistance to diabetes, hypertension, reproductive dysfunction, insomnia, and anxiety-related behavioral disorders [3-4].

Subsequent health derangements stemming from stress are often attributed to the deleterious influence of free radicals, which inflict tissue damage within the body [5]. The intriguing aspect lies in the inherent natural control of free radical formation by a cohort of beneficial compounds recognized as antioxidants [6]. Antioxidants have the capability to decrease molecular oxidation, and a well-balanced or supplemented diet rich in antioxidant sources is particularly crucial for sustaining overall well-being [7]. Through their ability to stabilize or counteract free radicals, antioxidants act as a protective barrier against damage to cells. Regrettably, insufficient availability of these antioxidants either in diets or supplements culminates in a gradual and incapacitating cellular degradation occasioned by unchecked free radicals [8].

The increasing global trend in the use of antioxidant treatments involving dietary supplements is now a widespread phenomenon and it represents a strategy to mitigate oxidative stress and resultant damage [9]. These dietary antioxidants are touted as instrumental for sustaining a healthy lifestyle and potentially extending lifespan [10]. These supplements encompass a wide range of constituents designed to fulfill vital nutritional requirements, spanning vitamins, minerals, amino acids, and herbal extracts, essentially forming an intermediary category between foods and medicinal agents. Emerging propositions posit that dietary antioxidants, including vitamins and non-nutrient counterparts like plant-derived flavonoids, exhibit the capacity to attenuate many of the deleterious consequences of oxidative stress [11-12].

Certain dietary supplements have additionally garnered attention for their purported ability to replenish the body's intrinsic and abundant antioxidant, reduced glutathione (GSH) [6]. Notably, these GSH-boosting dietary supplements, despite their limited empirical validation, are postulated to play a pivotal role in preventing various diseases [13], particularly those caused by free radicals and characterized by diminished GSH tissue reservoirs [14]. Among these, Cellgevity®, a dietary supplement, contains reduced glutathione precursor molecule Riboceine (D-ribose-L-cysteine), which has been shown to increase cellular transportation of cysteine, therefore increasing GSH levels. Riboceine has exhibited superior efficacy compared to other GSH enhancers [15].

Considering the convincing evidence supporting the importance of maintaining strong antioxidant defenses and the various uses of Cellgevity® in improving systemic and/or tissue-specific glutathione levels, our hypothesis suggests that Cellgevity® could enhance glucometabolic disorders triggered by stress by augmenting GSH reserves and alleviating oxidative damage. Consequently, we examined the glucometabolic potentials of Cellgevity® under stress conditions, using Sprague-Dawley rats as the experimental model.

MATERIALS AND METHODS

Animals: Twenty-four (24) male Sprague-Dawley rats (207±20g) were obtained and sustained on a regular chow and adequate water at our animal facility in controlled humidity (60% ± 5%) under a 12-hour light-dark cycle. All experimental procedures were ratified by the Institutional Research and Ethics Committee and conformed to the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health for studies involving experimental animals and the procedures as documented by Kilkenny *et al.* [16].

Experimental Designs: The rats were randomly distributed into four groups of six rats each. Group one- No Stress + Placebo (NSP) serve as negative control and was given distilled water as a placebo (same volume as cellgevity solution). Group two- No Stress + CellGevity (NSCG) served as a positive control and administered with Cellgevity® supplementation (30 mg/kg *b.w*). Groups three and four were exposed to restraint as well as cold stress. While group three: Stress + Placebo (STP) was given distilled water (*vol*/kg *b.w*), group 4: Stress + CellGevity (STCG) received Cellgevity® supplementation (30 mg/kg *b.w*).

Stress Protocol: This study employs a modified version of the restraint as well as cold stress protocol described by Paula-Freire et al. [17]. Cold restraint stress effectively imitates a physical and psychological stress state [18]. The animals were exposed to stress for the duration of 10

days. The immobilization (restraint) stress was performed in the morning via a cylindrical cage for 2 hours daily (8:00 h 10:00 h.) while the Cold pressure was applied for 2 hours in the evening (16:00 h 18:00 h).

Cellgevity Dietary Supplements: Cellgevity® (CG) dietary supplement used in this study was manufactured by Cornerstone Research and Development Inc, Ogden, Utah USA. Each capsule contained 21.25 mg of Vitamin C, 6.25 µg Selenium, 62.5 mg RiboCeine, and 290 mg proprietary blend. Cellgevity (CG) was prepared daily with distilled water for use in supplementation. All of the other chemical compounds utilized in this study were of excellent analytical standard. The CG supplementation began a week prior to the stress trials and was sustained for a period of 10 days. Animals were administered CG via the oral gavage after being dissolved in distilled water (vehicle) [19].

Oral glucose tolerance test (OGTT): On the fourteenth day of CG administration, all experimental rats were fasted for a period of twelve hours, and the fasting glucose level (FBG) value was set as the baseline blood glucose value for 0 minutes. Following that, the rats were given a gavage containing 2.0 g/kg of glucose after diluting it in distilled water, and FBG was measured after thirty, sixty, and 120 minutes of glucose ingestion. The value of the AUC (area under the curve) was computed.

Blood Collection: On the seventeenth day of CG administration, the rats were subjected to the two stress protocols concurrently over a period of two hours [17]. Following the final stress session, blood samples were collected via ocular puncture into heparinized and plain plastic tubes (which were allowed to clot for 30 minutes before centrifugation at 4°C for a period of 10 minutes at 10,000 revolutions per minute (rpm). Plasma and serum samples were thereafter stored in sealable polypropylene micro-centrifuge tubes at –20° C until assayed for serum corticosterone, plasma oxidative markers, serum insulin levels, serum liver enzymes, and other biochemical parameters.

Fasting insulin level, insulin sensitivity, and resistance indices: The fasting insulin level (FINS, µIU/mL) was determined using the ELISA kit, and the following were calculated.

Insulin sensitivity index (ISI)=Ln [(FBG dieFINS-1)]

Insulin resistance index (IRI)= (FBG um FINS)/22.5.

Corticosterone Levels: Corticosterone hormone is a marker of stress used to assess levels of stress. The ELISA assay was carried out with the use of a commercially available CORT kit (Elabscience Biotechnology Co., Ltd., China), which is a sensitive bioassay for determining the concentration of corticosterone in body fluids. The process was carried out according to the directions provided by the manufacturer.

Antioxidant enzymes and Oxidative markers: Activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and levels of malonaldehyde (MDA) were determined in both blood and tissue samples. As for the tissue samples, liver homogenates were prepared (immediately after dissection) from a known weight of the liver 10% tissues (w/v) in 0.05 M phosphate buffer, pH 7.4.

The procedure described by Van Dooran et al. [20] was used to calculate GSH activity. The procedure is based on the reaction of Ellman's 5, 5 'dithiobis (2-nitrobenzoic acid) DNTB) reagent with the GSH thiol group at pH 8.0 resulting in a yellow 5-thiol-2-

nitrobenzoate at 412nm. Sun and Zigman's [21] procedure was also used to determine SOD activity.

BIOCHEMICAL PARAMETERS

Liver Enzymes: The enzymes aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were all measured with a commercial kit and an automated analyzer (CobasMira, Roche Diagnostic Systems, Switzerland).

Lipid Profile: An automated biochemistry analyzer (BT 2000 Plus, Germany) was used to estimate total cholesterol (CHOL), triglycerides (TRIG), and high-density lipoprotein (HDL) using diagnostic kits for each one purchased from BioSystems® (S.A Costa Brava of Barcelona, Spain). Low-density lipoprotein was computed using the Freidewald formula [22].

Determination of Ferric Reducing Ability of Plasma (FRAP): Ferric ion reducing antioxidant power (FRAP) is an antioxidant capacity assay. The total antioxidant capacity (FRAP) was determined by the potential of the samples to decrease Fe+3 to Fe+2, which was subsequently chelated with TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) to generate the Fe+2-TPTZ deep blue complex [23]. The reaction was subsequently quantified using a spectrophotometer at a wavelength of 593 nm.

Determination of Glucose-6-Phosphate Dehydrogenase (G6PDH): The enzyme activity of G6PDH was measured using nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) production at a wavelength of 340 nm.

Alpha-amylase and alpha-glucosidase evaluation: This experiment was a modified method by McCue and Shetty (2004). The solutions were incubated at 25°C for 5 minutes before being halted after boiling for 2 minutes. The absorbance of the resultant p-nitro phenol (pNP) was measured at 405 nm with a spectrophotometer and was shown to be proportional to the enzyme activity. The alpha-glucosidase inhibitory assay incorporated acarbose as the reference drug. All experiments were carried out in triplicate.

Statistical analysis: GraphPad Prism 7.01 software (GraphPad Software, Inc.) was used for all data analyses. One-way ANOVA was used to compare the measurement data presented as mean ± standard error of the mean. Multiple comparisons were performed using the Students Newman-Kruel (SNK) method. The level of statistical significance was defined as a p-value of 0.05.

RESULTS

Effect of Cellgevity® on Blood Glucose level and Insulin Concentration: As indicated in figure 1a below, there was no significant difference (P>0.05) in the fasting blood glucose concentration in STP, NSCG and STCG rats (72.50 ± 9.55, 78.17 ± 6.56 and 59.00 ± 2.48) compared with NSP group (74.17 ± 9.55). However, the glucose concentration in STCG group (59.00 ± 2.48) was significantly lower ($p<0.05$) compared with STP and NSCG rats (72.50 ± 9.55, 78.17 ± 6.56). Figure 1b indicates that Cold restraint stress caused a significant ($p<0.05$) increase in insulin concentration in STP group when compared with the NSP (0.42 ± 0.01 vs 0.34 ± 0.02). However, STCG rats had significantly ($p< 0.05$) reduced concentration of insulin compared with STP rats (0.36 ± 0.01 vs 0.42 ± 0.01) (Figure 1b).

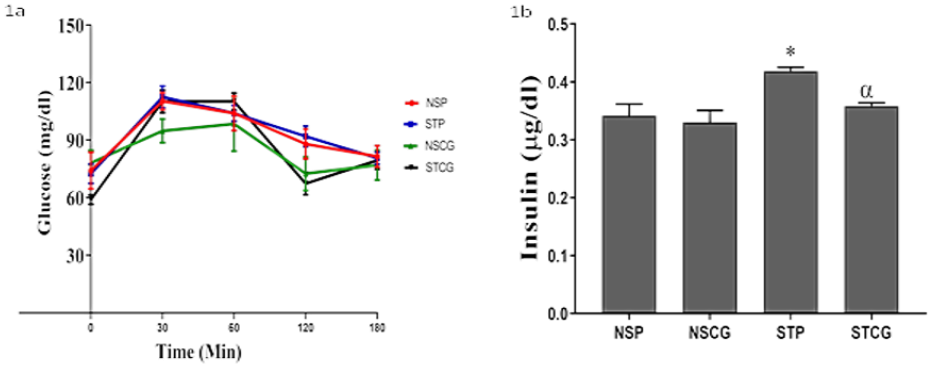


Figure 1a. Effect of Cellgevity® on blood glucose level in cold-restraint rats during glucose tolerance test.

Figure 1b. Effect of Cellgevity® on serum insulin concentration in cold-restraint rats. Value expressed as mean ± SEM, n= 6 per group, ∗p < 0.05 *vs* NSP, α p< 0.05 *vs* STP (one-way ANOVA followed by SNK post hoc test).

Effect of Cellgevity® on Glucose Tolerance and Insulin Sensitivity (using HOMA-IR): The blood glucose curve during the glucose tolerance test in stressed (STP, STCG) and no stress animals (NSP, NSCG) is depicted in figure 2a. Results from the GTT test showed no significant difference (p>0.05) in the glucose tolerance of both stressed (STP, STCG) when compared with no stress animals (NSP, NSCG) as indicated by a similar AUC glucose (Figure 2a). While in Fig 2b, Cellgevity® produced no significant difference ($p > 0.05$) in the HOMA-IR index between NSCG and NSP. However, HOMA-IR was significantly increased in STP (1.34 ± 0.04) compared with control rats (1.12 ± 0.04). Conversely, HOMA-IR in STCG group (0.94 ± 0.06) was significantly reduced compared with both NSP and STP (1.12 ± 0.04, 1.34 ± 0.04) (Figure 2b).

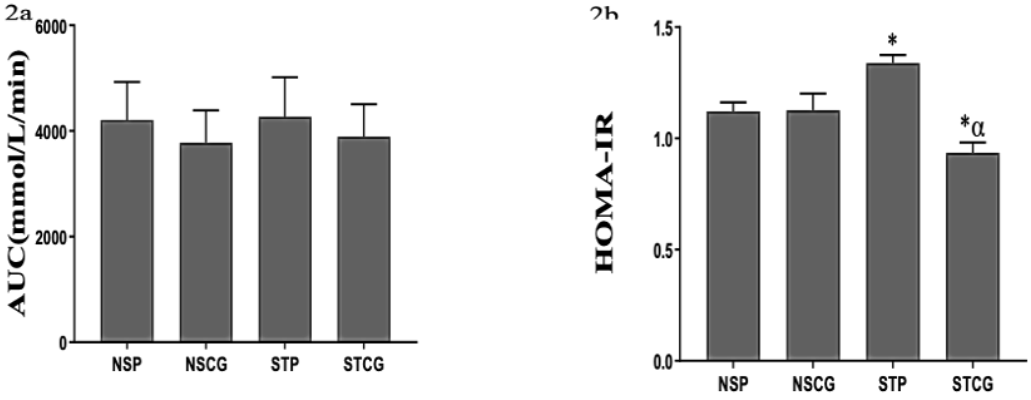


Figure 2a. Effect of Cellgevity® on AUC_{OGTT} level in cold-restraint rats.

Figure 2b. Effect of Cellgevity® on HOMA-IR indices level in cold-restraint rats. Values expressed as mean ± SEM, n= 6 per group, ∗p < 0.05 *vs* NSP, α p< 0.05 *vs* STP (one-way ANOVA followed by SNK post hoc test).

Figure 3 shows the corticosterone level in rats exposed to cold stress and their control group. CORT level in STP and STCG groups was significant higher (P<0.05) compared with control group (NSP) indicating elevated stress level in STP and STCG. However, administration of Cellgevity® caused a significant reduction (p<0.05) in CORT level of

STCG compared to STP (15.52 ± 0.27 vs 20.05 ± 0.36) (Figure 3).

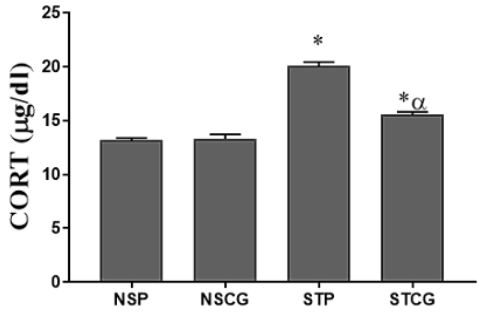


Figure 3. Effect of Cellgevity® on corticosterone level in cold-restraint rats. Values are expressed as mean ± SEM, n= 6 per group, ∗p < 0.05 *vs* NSP, α p< 0.05 *vs* STP (one-way ANOVA followed by SNK post hoc test).

Effect of Cellgevity® on corticosterone (CORT) level.

Effect of Cellgevity® on oxidative stress and antioxidant markers: The effect of cold restraint stress was examined on serum oxidative stress and antioxidant biomarkers in male Sprague-Dawley rats. Figure 4 (a-d) shows the effect of cold restraint stress on SOD and CAT levels. The STP group showed a significant decrease in CAT level compared with NSP (Control) group. Meanwhile the STP group had a significant higher MDA level, while the GSH level showed no significant difference compared with control (NSP) rats. STCG rats had significant higher levels of SOD and CAT compared with NSP and STP rats. However, GSH level in STCG group is within the range of STP and NSP rats. STCG rats had significant higher MDA level compared with NSP but a significantly lower MDA level compared with STP.

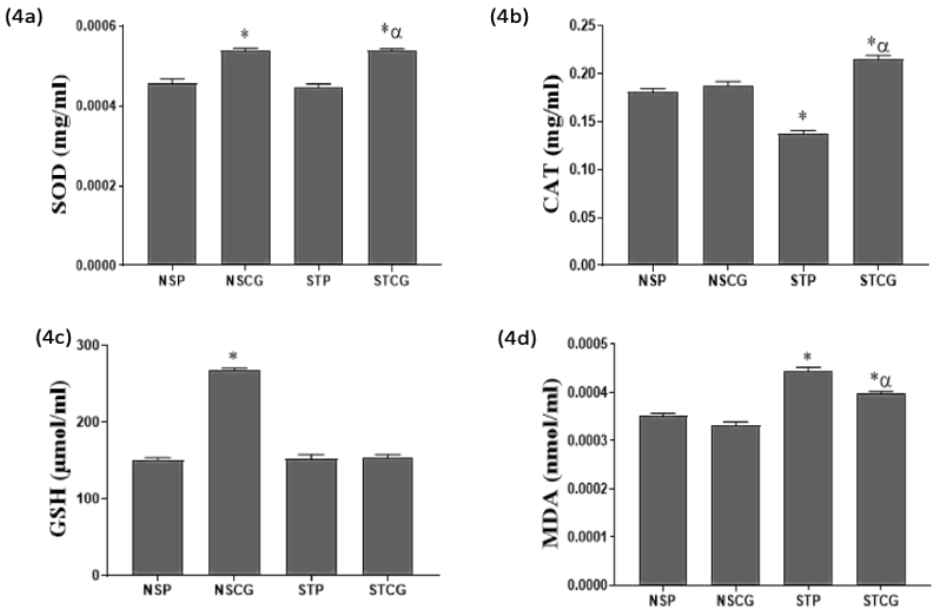


Figure 4 a-d. Effect of Cellgevity® (a) SOD, (b) CAT, (c) GSH, (d) MDA in cold-restraint rats. Values expressed as mean ± SEM, n= 6 per group, ∗p < 0.05 *vs* NSP, α p< 0.05 *vs* STP (one-way ANOVA followed by SNK post hoc test).

3.05 Effect of Cellgevity® on liver enzymes : In the plasma, the AST level of the cold-restraint (STP) and Cellgevity® (STCG) groups were similar to the control (NSP). Conversely, both cold-restraint (STP) and

Cellgevity® (STCG) groups appear to significantly cause a higher level of ALT and ALP as seen compared with NSP.

Table 1. Effect of Cellgevity® on liver enzyme in cold-restraint rats

Parameters	NSP	NSCG	STP	STCG
AST	58.17±0.31	62.17±0.31	64.20±0.37	58.40±0.68
ALT	16.40±0.51	16.50±0.29	23.00±0.45*	23.50±0.29*
ALP	20.40±0.24	27.50±0.29*	26.80±0.37*	27.83±0.31*

Values expressed as mean ± SEM, n= 6 per group, ∗p < 0.05 *vs* NSP (one-way ANOVA followed by SNK post hoc test).

Effect of Cellgevity® on Lipid Profile: We examined the effect of Cellgevity® on serum lipid profile in cold restraint stressed male Sprague-Dawley rats. According to Table 2 below, rats exposed to cold restraint stress (STP) had similar CHOL and LDL levels compared with NSP rats. However, the HDL level in STP rats increased significantly ($p<0.05$) compared with NSP, but the TRIG level showed a significant decrease ($p<0.05$). Administration of Cellgevity® profoundly alters the lipid profile in STCG rats as significant reduction was observed in all the lipid profile parameters when compared with NSP and STP groups. Furthermore, NSCG rats had significantly lower CHOL and TRIG compared with control. The HDL level in the NSCG group was similar to control, while LDL level was significantly higher.

Table 2. Effect of Cellgevity® on lipid profile index in cold-restraint rats

Parameters	NSP	NSCG	STP	STCG
Chol	2.07±0.05	1.77±0.03*	1.98±0.05	1.68±0.04*α
TRIG	0.98±0.02	0.78±0.02*	0.68±0.02*	0.55±0.03* α
HDL	1.02±0.02	0.95±0.02	1.15±0.02*	0.90±0.04*α
LDL	0.65±0.03	0.86±0.04*	0.68±0.02	0.55±0.03*α

Values expressed as mean ± SEM, n= 6 per group, ∗p < 0.05 *vs* NSP, α p< 0.05 *vs* STP (one-way ANOVA followed by SNK post hoc test).

Effect of Cellgevity® on FRAP and G6PDH level: Figure 5a shows the level of FRAP after cold restraint stress exposure in rats. Cold-restraint stress (STP) significantly elevated FRAP level in STP group compared with control (NSP). However, following the administration of Cellgevity®, FRAP level was significantly reduced ($p<0.05$) in STCG rats compared with NSP and STP. FRAP level was also significantly reduced in NSCG rats compared with

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.

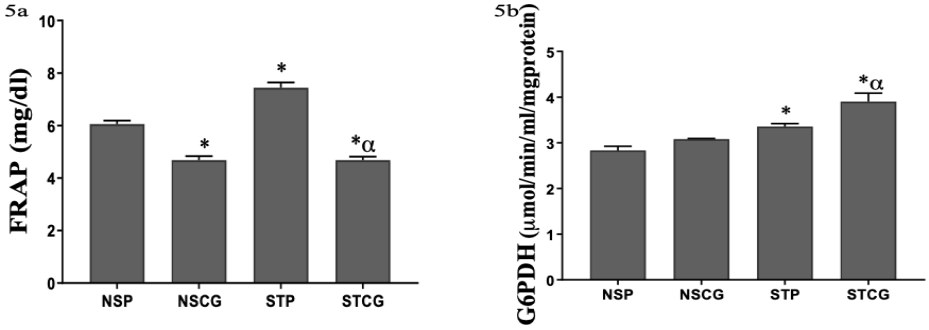


Figure 5a. Effect of Cellgevity® on FRAP level in cold-restraint rats.

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* NSP, α p< 0.05 *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.

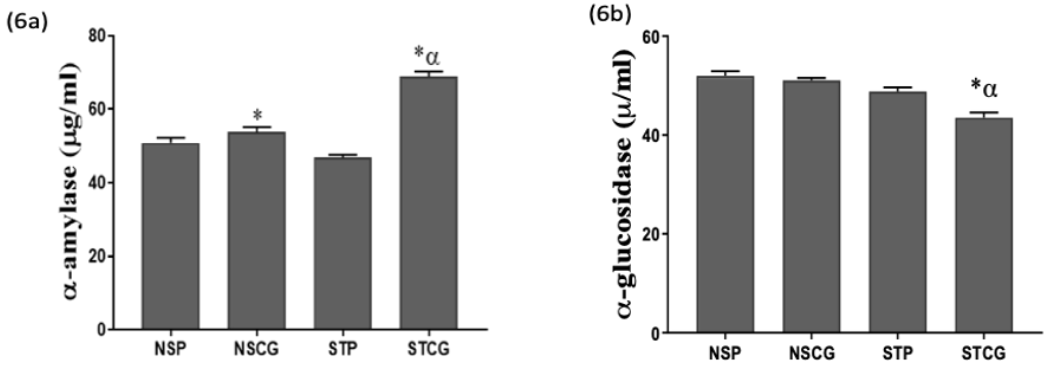


Figure 6a. Effect of Cellgevity® on α-amylase level in cold-restraint rats.

Figure 6b. Effect of Cellgevity® on α-glucosidase level in cold-restraint rats. Values expressed as mean ± SEM, n= 6 per group, ∗p < 0.05 *vs* NSP, α 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.

Acknowledgement: The authors will like to appreciate the support and services of the laboratory of Dr. Morakinyo for providing us reagents and equipment used for this research work.

Funding: This research received no funding from any agency.

REFERENCES

1. Chu B, Marwaha K, Sanvictores T, Ayers D. Physiology, Stress Reaction. 2022 Sep 12. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.
2. Stairs DJ, Bardo MT. Neurobehavioral effects of environmental enrichment and drug abuse vulnerability. Pharmacology Biochemistry and Behaviour. 2009;92(3):377-82. DOI: https://doi.org/10.1016/j.pbb.2009.01.016
3. Deding U, Ejlskov L, Grabas MP, Nielsen BJ, Torp-Pedersen C, Bøggild H. Perceived stress as a risk factor for peptic ulcers: a register-based cohort study. Biomedical Medical Central Gastroenterology. 2016;16(1):140. DOI: https://doi.org/10.1186/s12876-016-0554-9
4. Morakinyo AO, Samuel TA, Awobajo FO, Adekunbi DA, Olatunji IO, Binibor FU, Oni AF. Adverse effects of noise stress on glucose homeostasis and insulin resistance in Sprague-Dawley rats. Heliyon. 2019;5(12): e03004 DOI: https://doi.org/10.1016/j.heliyon.2019.e03004
5. Terraneo L, Paroni R, Bianciardi P, Giallongo T, Carelli S, Gorio A, Samaja M. Brain adaptation to hypoxia and hyperoxia in mice. Redox Biology. 2017; 11:12-20. DOI: https://doi.org/10.1016/j.redox.2016.10.018
6. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews. 2010;4(8):118-26. DOI: https://doi.org/10.4103/0973-7847.70902
7. Danik Martirosyan, Mohammad Reza Ashoori, Alireza Rezaeinezhad , Fahimeh Jahanbakhshi , Shaghayegh Pezeshki , Afsaneh Seyed Mikaeili , Saham Alkhamis, and Hossein Mirmiranpour. Effects of quercetin and low-level laser on oxidative and inflammatory factors among patients with type 2 diabetes mellitus and mild cognitive impairment. Bioactive Compounds in Health and Disease 2022;5(1):1-12. DOI: https://doi.org/10.31989/bchd.v5i1.877
8. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Europian journal of Medicinal Chemistry. 2015;5;97:55-74. DOI: https://doi.org/10.1016/j.ejmech.2015.04.040
9. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, Arrigo T. Oxidative stress in obesity: a critical component in human diseases. International Journal of Molecular Science. 2014;16(1):378-400. DOI: https://doi.org/10.3390/ijms16010378
10. Salminen A, Kaarniranta K, Kauppinen A. Inflammaging: disturbed interplay between autophagy and

inflammasomes. Aging (Albany NY). 2012;4(3):166-75. DOI: https://doi.org/10.18632/aging.100444

11. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos University of Medical Journal. 2012;12(1):5-18. DOI: https://doi.org/10.12816/0003082
12. Halliwell B. Biochemistry of oxidative stress. Biochemical Society Transaction. 2007;35(Pt 5):1147-50. DOI: https://doi.org/10.1042/BST0351147
13. Fang, Y. Z., Yang, S., & Wu, G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872-879. DOI: https://doi.org/10.1016/S0899-9007(02)00916-4
14. Turk SR, Shipman C Jr, Drach JC. Selective inhibition of herpes simplex virus ribonucleoside diphosphate reductase by derivatives of 2-acetylpyridine thiosemicarbazone. Biochemical Pharmacology. 1986;35(9):1539-45. DOI: https://doi.org/10.1016/0006-2952(86)90122-x
15. Oz HS, Chen TS, Neuman M. Nutrition intervention: a strategy against systemic inflammatory syndrme. Journal of parenteral Enteral Nutrition. 2009;33(4):380-9. DOI: https://doi.org/10.1177/0148607108327194
16. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biology. 2010;8(6): e1000412. DOI: https://doi.org/10.1371/journal.pbio.1000412
17. Neculicioiu VS, Colosi IA, Costache C, Toc DA, Sevastre-Berghian A, Colosi HA, Clichici S. Sleep Deprivation-Induced Oxidative Stress in Rat Models: A Scoping Systematic Review. Antioxidants (Basel). 2023;12(8):1600. DOI: https://doi.org/10.3390/antiox12081600
18. Klenerová, V., Krejcí, I., Sida, P., & Hlinák, Z.. A comparison of the effects of various stressors on the release of 5-HT and NA in the frontal cortex, amygdala, and hypothalamus of rats. Neurochemical Research. 2003;28(2):257-261. DOI: https://doi.org/10.1371/journal.pone.0029988
19. Falana B, Adeleke O, Orenolu M, Osinubi A, Oyewopo A. Effect of D-ribose-L-cysteine on aluminum induced testicular damage in male Sprague-Dawley rats. JBRA Assisted Reproduction. 2017;21(2):94-100. DOI: https://doi.org/10.5935/1518-0557.20170023
20. Couto N, Wood J, Barber J. The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free Radical Biology and Medicine. 2016; 95:27-42. DOI: https://doi.org/10.1016/j.freeradbiomed.2016.02.028
21. Sun, M. and Zigma, S. An Improved Spectrophotometer Assay of Superoxide Dismutase Based on Epinephrine Antioxidation. Analytical Biochemistry. (1978); 90:81-89. DOI: https://doi.org/10.1016/0003-2697(78)90010-6
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502. DOI: https://doi.org/10.1093/clinchem/18.6.499
23. Diniz Vilela D, Gomes Peixoto L, Teixeira RR, Belele Baptista N, Carvalho Caixeta D, Vieira de Souza A, Machado HL, Pereira MN, Sabino-Silva R, Espindola FS. The Role of Metformin in Controlling Oxidative Stress in Muscle of Diabetic Rats. Oxidative Medicine and Cellular Longevity. 2016;2016:6978625. DOI: https://doi.org/10.1155/2016/6978625
24. Mizuma H, Tanaka M, Nozaki S, Mizuno K, Tahara T, Ataka S, Sugino T, Shirai T, Kajimoto Y, Kuratsune H, Kajimoto O, Watanabe Y. Daily oral administration of crocetin attenuates physical fatigue in human subjects. Nutr Res. 2009;29(3):145-50. DOI: https://doi.org/10.1016/j.nutres.2009.02.003
25. Bryan NS, Calvert JW, Elrod JW, Gundewar S, Ji SY, Lefer DJ. Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. Proceeding of National Academy Science U S A. 2007;104(48):19144-9. DOI: https://doi.org/10.1073/pnas.0706579104
26. Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN, Lakshminarasaiah U, Gopas J, Nishigaki I. Antioxidants and human diseases. Clinica Chimica Acta. 2014;436:332-47. DOI: https://doi.org/10.1016/j.cca.2014.06.004
27. Thomas DD, Corkey BE, Istfan NW, Apovian CM. Hyperinsulinemia: An Early Indicator of Metabolic Dysfunction. Journal of Endocrine Society. 2019;3(9):1727-1747. DOI: https://doi.org/10.1210/js.2019-00065
28. Dungan KM, Braithwaite SS, Preiser JC. Stress hyperglycaemia. Lancet. 2009 23;373(9677):1798-807. DOI: https://doi.org/10.1016/S0140-6736(09)60553-5
29. Stuvoll, M., Dokken, B. B., Hjermann, I., and Hoieggen, A.. Acute glucose and lipid-lowering effects of captopril in patients with hypertension and type 2 diabetes. Diabetes, Obesity and Metabolism. (2000);2(5):325-332. DOI: https://www.doi.org/10.1016/0002-9343(88)90584-0
30. Das AB, Nauser T, Koppenol WH, Kettle AJ, Winterbourn CC, Nagy P. Rapid reaction of superoxide with insulin-tyrosyl

radicals to generate a hydroperoxide with subsequent glutathione addition. Free Radical Biology Medicine. 2014; 70:86-95. DOI: https://www.doi.org/10.1016/j.freeradbiomed.2014.02.006

31. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. 2001;414(6865):799-806. DOI: https://doi.org/10.1038/414799a
32. Mustafa T, Jiang SZ, Eiden AM, Weihe E, Thistlethwaite I, Eiden LE. Impact of PACAP and PAC1 receptor deficiency on the neurochemical and behavioral effects of acute and chronic restraint stress in male C57BL/6 mice. Stress. 2015;18(4):408-18. DOI: https://doi.org/10.3109/10253890.2015.1025044
33. Ozbaki J, Goudarzi I, Salmani ME, Rashidy-Pour A. Acute stress does not affect the impairing effect of chronic stress on memory retrieval. Iran Journal of Basic Medical Science. 2016;19(7):763-71. DOI: https://doi.org/10.22038/ijbms.2016.7362
34. Sarkhel S. Kaplan and Sadock's Synopsis of Psychiatry: Behavioral Sciences/Clinical Psychiatry, 10th edition. Indian Journal of Psychiatry. 2009;51(4):331. DOI: https://doi.org/10.4088/JCP.09bk05044
35. Zhang S, Xu Z, Gao Y, Wu Y, Li Z, Liu H, Zhang C. Bidirectional crosstalk between stress-induced gastric ulcer and depression under chronic stress. PLoS One. 2012;7(12):e51148. DOI: https://doi.org/10.1371/journal.pone.0051148
36. Arts RJ, Blok BA, Aaby P, Joosten LA, de Jong D, van der Meer JW, Benn CS, van Crevel R, Netea MG. Long-term in vitro and in vivo effects of γ-irradiated BCG on innate and adaptive immunity. Journal of Leukocyte Biology. 2015;(6):995-1001. DOI: https://doi.org/10.1189/jlb.4MA0215-059R
37. Sahin K, Orhan C, Tuzcu M, Ali S, Sahin N, Hayirli A. Epigallocatechin-3-gallate prevents lipid peroxidation and enhances antioxidant defense system via modulating hepatic nuclear transcription factors in heat-stressed quails. Poultry Science. 2010 (10):2251-8. DOI: https://doi.org/10.3382/ps.2010-00749
38. Piato AL, Detanico BC, Linck VM, Herrmann AP, Nunes DS, Elisabetsky E. Anti-stress effects of the "tonic"Ptychopetalum olacoides (Marapuama) in mice. Phytomedicine. 2010;17 (3-4):248-53. DOI: https://doi.org/10.1016/j.phymed.2009.07.001
39. Sharma K, Akre S, Chakole S, Wanjari MB. Stress-Induced Diabetes: A Review. Cureus Journal of Medical Science. 2022;14(9):e29142. DOI: https://doi.org/10.7759/cureus.29142
40. Damm TW, Kramer DJ. The Liver in Critical Illness. Critical Care Clinics. 2016;32(3):425-38. DOI: https://doi.org/10.1016/j.ccc.2016.02.002
41. Song R, Hu W, Cheng R, Zhao Y, Qin W, Li X, Zhu Y, Gan L, Liu J. Association Between Circulating Levels of C1q/TNF-Related Protein-9 and Type 2 Diabetes Mellitus: A Systematic Review and Meta-analysis. Journal of Clinical Endocrinology and Metabolism. 2023;108(10):2728-2738. DOI: https://doi.org/10.1210/clinem/dgad172
42. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 2003;423(6941):762-9. DOI: https://doi.org/10.1038/nature01705
43. Michelson D, Galliven E, Hill L, Demitrack M, Chrousos G, Gold P. Chronic imipramine is associated with diminished hypothalamic-pituitary-adrenal axis responsivity in healthy humans. Journal of Clinical Endocrinology and Metabolism. 1997;82(8):2601-6. DOI: https://doi.org/10.1210/jcem.82.8.4172
44. Gong L, Feng D, Wang T, Ren Y, Liu Y, Wang J. Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. Food Science and Nutrition. 2020;8(12):6320-6337. DOI: https://doi.org/10.1002/fsn3.1987