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IN VITRO EVALUATION OF THE ANTIGLYCATION AND ANTIOXIDANT POTENTIAL OF THE DIETARY SUPPLEMENT L-CITRULLINE

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Diabetes mellitus (DM) represents a significant global public health concern. It is a metabolic condition characterized by abnormal glucose levels in the bloodstream, known as hyperglycemia. This condition arises due to irregular insulin secretion, defective insulin receptivity, or a combination of both factors. The primary contributors to diabetic complications are protein glycation and oxidative stress resulting from chronic hyperglycemia.

The aim. *The increasing incidence of diabetes mellitus has prompted a quest for a novel, cost-effective, and efficacious medication. The objective of the study generally intends to explore and investigate the antiglycation and antioxidant potential of the dietary supplement L-Citrulline*

Materials and methods. *A two-reaction model system was carried out to study and monitor the inhibitory impact of the dietary supplement L-Citrulline against advanced glycation end products (AGEs) formation. This system involved the in vitro glucose bovine serum albumin (BSA-glucose assay) and methylglyoxal bovine serum albumin (BSA-MGO assay). The antioxidant activity of the supplement was assessed by measuring its capacity to chelate metal ions and scavenge reactive oxygen species. The iron chelating activity was evaluated through absorbance measurements, while fluorescence measurements were employed for the remaining assays.*

Results. *According to the findings of the antiglycation assays, it was observed that the dietary supplement L-Citrulline demonstrated inhibitory properties against the development of advanced glycation end products (AGEs) in the BSA-Glucose model at a concentration of 100 ppm. The degree of inhibition with respect to glycation was ascertained to be 52.19±0.39 % through observation. The BSA-MGO model has exhibited inhibitory properties with an observed activity of 49.64±0.27 % at 100ppm concentration with respect to glycation. On the other hand, the supplement demonstrates antioxidant characteristics through the chelation of Fe ions, leading to a percentage difference in activity of 68.58±0.45 % compared to the control at 100 ppm. The utilization of Glucolypotoxixity (GLT) media during the reactive oxygen species assay yielded a significant rise of 173.48±9.37 % in the reactive species levels compared to the control, with statistical significance. The addition of 10 mM dietary supplement L-Citrulline resulted in a noteworthy reduction of 98.42±5.04 % in the escalation. Therefore, it can be deduced that utilizing L-Citrulline as a dietary supplement exhibits potential for its therapeutic applications in eliminating reactive oxygen species (ROS) within skeletal muscle cells.*

Conclusion. *The study results suggest that the dietary supplement L-Citrulline has demonstrated inhibitory capabilities against glycation at varying concentration levels. Furthermore, it was noted to exhibit significant efficacy in both sets of antioxidant tests. Therefore, the supplement exhibits potential in the treatment of diabetes mellitus*

Keywords: *Type 2 Diabetes, hyperglycemia, in vitro, dietary supplement L-Citrulline*

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1. Introduction

There has been a substantial increase in the number of individuals diagnosed with diabetes. In the year 2021, it is estimated that diabetes mellitus has impacted approximately 537 million adults, resulting in an estimated 6.7 million fatalities worldwide. It is anticipated that there will be a substantial rise of 10.4 % in the population impacted, with a projected 643 million individuals in 2030 and 783 million in 2045 [1]. Diabetes mellitus is a medical condition marked by elevated glucose levels in the blood, and individuals affected by this condition are susceptible to developing chronic complications [2, 3]. High blood glucose levels, known as hyperglycemia, are a significant factor in the onset of these complications due to heightened protein glycation. Protein glycation occurs through a nucleophilic addition reaction between a carbonyl group originating from a reducing sugar and a free amino group present in a protein, which leads to the creation of Schiff bases that are freely reversible and subsequently rearranged to form more stable Amadori products. In the presence of transition metals and oxygen, glucose and Amadori products undergo autoxidation, creating free radicals through autoxidative glycation and glycoxidation, respectively [4–6]. Subsequently, the free

radicals induce harm to biomolecules within the human body [7].

The reaction between advanced glycation end products (AGEs) and cellular receptors for AGEs may produce proinflammatory molecules and oxidative stress. The long-term complications of diabetes mellitus are attributed to AGE accumulation in tissues and oxidative stress [8]. Several studies on diabetes have indicated that hyperglycemia is associated with oxidative stress through glucose autooxidation and a disruption of the electron transport chain. The catalysis of glucose autooxidation by transition metals has the potential to produce ketoaldehyde and superoxide radicals ($O2\cdot^{-}$). The Fenton reaction facilitates the conversion of superoxide radical to hydroxyl radical ($OH\cdot$) [9]. Accelerated oxidation may lead to cellular damage and activation of specific signalling pathways, such as the nuclear factor-κB (NF-κB), which can trigger the production of proinflammatory cytokines [9, 10]. Protein glycation is a fundamental molecular mechanism underlying diabetic complications arising from prolonged hyperglycemia. Consequently, the suppression of advanced glycation end products (AGEs) represents a different approach to managing diabetes that is not reliant on regulating blood glucose levels. This strategy could prove beneficial in averting or alleviating specific diabetic complications [11].

Numerous synthetic inhibitors of advanced glycation end products (AGEs) have been discovered recently, including aminoguanidine (AG), the most widely recognized synthetic prodrug. These inhibitors are effective in preventing the formation of AGEs. Nonetheless, the practical applications of these substances are restricted due to their toxicity and significant adverse effects [9]. Furthermore, specific inhibitors of advanced glycation end products (AGEs) have been found to contribute to pyridoxal sequestration, resulting in vitamin B6 deficiency among diabetic patients [9].

Natural resources like plants have always been on the frontline in formulating alternative medicine. However, few existing studies uphold the potential of amino acid-based supplements in therapeutic and clinical applications. As one of the many supplementations, L-Citrulline is a non-essential amino acid produced by the liver that is proposed to be a potential treatment for Type 2 Diabetes Mellitus (T2DM) [12, 13]. Maintenance of normal endothelial function, hepatic and peripheral insulin sensitivity, and modulation of systemic glucose metabolism is a function of nitric oxide (NO), which is disrupted in the setting of T2DM due to deficit NO synthase [14, 15]. There are also findings that uphold L-Citrulline possesses antioxidant effects because of its ability to decrease the production of reactive oxygen species (ROS) caused by oxidative stress [16]. L-Citrulline is also investigated to have anti-inflammatory factors, which are inferred to impact the healing of chronic hyperglycemia positively [17]. Furthermore, L-Citrulline is eyed by many experts to be effective against diabetes because of its effect on lipid profile, inflammation, and glycemic control [18–20].

The reported effects of L-Citrulline suggest its edge in prevention and safe treatment for DM and even other diseases. However, despite the promising results of studies about L-Citrulline, literature demonstrating the capacity of this amino acid is still lacking. In this research, the focus was on the utilization of the dietary supplement L-Citrulline. This supplement is chosen due to its popularity as an alternative for individuals seeking various health benefits. Notably, among those who commonly incorporate this supplement into their regimen are individuals aiming to enhance their muscle-building efforts. Hence, the purpose of this study is to evaluate further the dietary supplement L-Citrulline's antiglycation and antioxidant in the hope of conveying new information and reinforcing claims of past research on the potential of the supplement in diabetes management.

2. Planning (methodology) of research

1. Assess the dietary supplement L-Citrulline as a potential antiglycation agent by evaluating its ability to inhibit the formation of advanced glycation and end products (AGEs) using the BSA-MGO and the BSA-Glucose model systems.

2. Investigate the antioxidant capacity of the dietary supplement L-Citrulline through one or more of the following assays: iron binding ability and its effects towards glucolipotoxic free radicals or reactive species using C2C12 muscle cells.

3. Materials and methods

3. 1. Sample preparation

Dietary supplement L-Citrulline drug (Manufactured & Quality Tested by NOW FOODS) was pulverized by mortar and pestle, and the sample was dissolved in double-distilled water. All reagents were in analytical reagent grade. Sample solutions in various concentrations (25, 50, 75, 100 ppm) were prepared through serial dilution using distilled water. All analyses were done in n=3 or more independent trials.

3. 2. Determination of antiglycation activity

The AGE assay uses fluorescence spectroscopy to monitor the inhibitory effect of the presence or absence of a substance against glycation. The two reaction model systems, BSA Glucose and BSA-MGO were used to determine antiglycation activity in the dietary supplement L-Citrulline.

BSA-Glucose model system preparation.

Different solution preparations of BSA (10 mg/mL) and glucose (90 mg/mL) were prepared in phosphate buffer (pH 7.4). Then, in a 10 mL test tube, 0.5 mL of the previous-prepared supplement drug L-Citrulline was combined with 0.5 mL of BSA and 0.5 mL of glucose solution. A blank solution was prepared using 0.5 mL of phosphate buffer, 0.5 mL of BSA solution, and 0.5 mL of glucose solution. On the other hand, 0.5 mL of Aminoguanidine (AG) solution (1 mol/L) was combined with 0.5 mL of BSA and 0.5 mL of glucose to prepare a positive control. In order to stop the growth of microbes, the tested solution additionally included 100 µL of 0.01 % NaN_3. The tubes were covered and cultured in a temperature-controlled incubator for seven days at 37 °C in the dark [21].

BSA-MGO model system preparation.

Separate solutions of BSA (2 mg/mL) and MGO (400 mg/mL) were dissolved in phosphate buffer (pH 7.4).

The remaining steps followed the same protocol as for the BSA-glucose model. Seven additional days at 37 °C in complete darkness served as the incubation period [21].

Measurement of AGE Fluorescence.

After an appropriate incubation procedure, the fluorescence of AGEs was measured using a ClarioStar microplate reader at excitation and emission wavelengths of 360 nm and 420 nm for BSA-glucose and 340 nm and 420 nm for BSA-MGO. For each set, the AGE Formation fluorescence wrt glycated by supplement was calculated using triplicate samples [21]. The equation below was utilized.

$$\%\ AGE\ Relative\ to\ Glycated = \left(\frac{A_{sample}}{A_{control}}\right) * 100, \quad (1)$$

where $A_{control}$ is the corrected absorbance of the control reaction (glycated), and A_{sample} is the corrected absorbance in the presence of a supplement.

3. 3. Screening of the antioxidant activity

Determination of metal chelating activity.

In order to measure metal chelating activity, 0.2 mL of 0.1 mM $FeSO_4$ (and 0.4 mL of 0.25 mM ferrozine were added to 0.2 mL of the supplement at varying concentrations. After 10 minutes of room-temperature incubation, the absorbance of the mixture was measured at 562 nm. Ascorbic acid was used as a positive control. Using the following formula, chelating activity absorbance concerning the control was determined [22].

$$\%\ Change\ Metal\ Chelating\ wrt\ Control = \left(\frac{A_{sample}}{A_{control}}\right) * 100, \quad (2)$$

where $A_{control}$ is the corrected absorbance of the control reaction (without the sample), and A_{sample} is the corrected absorbance in the presence of a supplement.

Reactive Species Detection Assay: Preparation of Healthy Control and Glucolipotoxicity Media in C212 cell.

TheC2C12cellline(ECACC 91031101) utilized in this study was procured from ChemoScience Philippines. Differentiated C2C12 myoblasts were subjected to incubation in either control or GLT media, which consisted of DMEM supplemented with 28 mM glucose, 200 µM Palmitic acid, and 200 µM Oleic acid for five days. The media was replaced on the 3rd day, followed by a final incubation of 1 hour with or without 10 mM supplement L-Citrulline, added to new experimental condition media. After each condition, a concentration of 20 µM of DCFDA was introduced and incubated for 1 hour. Afterwards, the cells were washed three times in PBS and then loaded with 20 µM DCFDA for an hour. Fluorescence with excitation occurring at 495 nm and emission occurring at 530 nm was used to detect radical species. ROS was expressed as a percentage change relative to control in all cases [23].

$$\%\ Change\ ROS\ scavenging\ wrt\ Control = \left(\frac{A_{sample}}{A_{control}}\right) * 100, \quad (3)$$

where $A_{control}$ is the corrected absorbance of the control reaction (without the sample), and A_{sample} is the corrected absorbance in the presence of a supplement.

3. 4. Statistical analysis

The outcomes were presented as the average value±standard error of the mean (SEM), based on a minimum of three independent experiments. The statistical method of one-way analysis of variance (ANOVA) was utilized to analyze data sets that consisted of more than two conditions. In instances where the results indicated a significant difference (p<0.05), a post hoc analysis was conducted using the Dunnett test. The process of assessing the data was executed through the utilization of the statistical software GraphPad Prism 9.0.0.

4. Results

4. 1. Antiglycation activity of supplement L-Citrulline

BSA-Glucose.

In evaluating AGE inhibition, two fluorescence-based model systems were utilized. The AGEs from glucose and MGO were seen using the BSA-glucose systems and BSA-methylglyoxal (MGO), respectively. The positive control used in the study was Aminoguanidine (AG), a pharmaceutical agent employed in managing diabetic complications. Highlighted graphically in Fig. 1, it demonstrates that the dietary supplement L-Citrulline BSA-Glucose assay findings show a dose-dependent response.

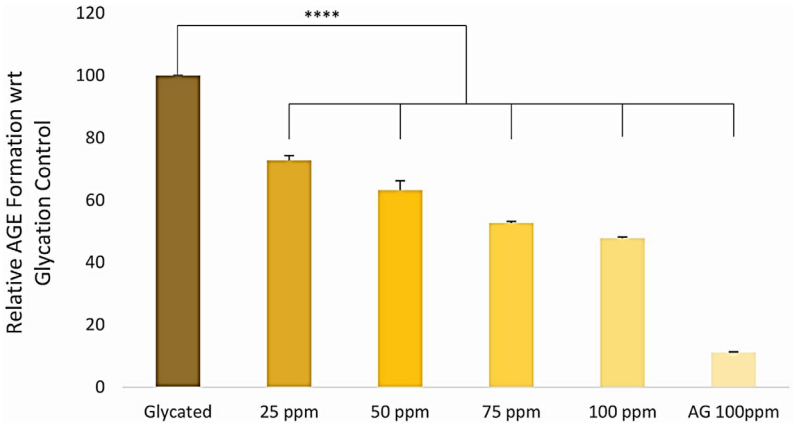

Fig. 1. The Antiglycation (BSA-Glucose) effect of dietary supplement L-Citrulline (25 ppm to 100 ppm) and AG (100 ppm) wrt glycated sample: **** – p<0.0001 wrt control; Dunnett's Test); n=3 independent trials

The study investigated the impact of the dietary supplement L-Citrulline on inhibiting advanced glyca-

tion end product (AGE) formation. The findings indicate that the dietary supplement L-Citrulline could impact the binding of glucose to bovine serum albumin (BSA), as evidenced by a more significant inhibition of glucose binding to BSA at higher supplement concentrations. The dose-dependent response suggests that added concentrations of the supplement may be linked to increased inhibition of glucose attaching to BSA, thereby implying a heightened potential of the supplement to interact with glucose molecules and hinder their binding to BSA.

The results revealed that at concentrations ranging from 100 to 25 ppm, dietary supplement L-Citrulline demonstrated a notable inhibition of AGE formation, with the percentage difference ranging from 52.19±0.39 % to 27.24±1.54 %. In comparison, the reference standard at a concentration of 100 ppm exhibited a higher inhibition activity of 88.89±0.22 %. These findings suggest that the dietary supplement L-Citrulline and the reference standard possess inhibitory potential across the studied concentration range, emphasizing their ability to counteract AGE formation.

BSA-MGO.

BSA-MGO is the second method used in the current study. During the intermediate phase of protein glycation, the glucose degradation and oxidation process results in the formation of Methylglyoxal (MGO), a notable α-dicarbonyl compound. Methylglyoxal (MGO) exhibits a higher propensity to react with amino groups present in proteins, thereby forming advanced glycation end products (AGEs).

As mentioned earlier, the current investigation utilized the dietary supplement L-Citrulline at varying concentrations to evaluate their inhibitory impact on the model. Shown in Fig. 2 depicts the percentage of inhibitory activity exhibited by the dietary supplement L-Citrulline and depicts a dose-dependent response. The inhibitory activity was observed across all supplement concentrations, with the highest prevention at 49.64±0.27 % being recorded at 100 ppm. The 75, 50, and 25 (ppm) concentrations were associated with per cent differences of 40.92±2.99 %, 36.19±5.76 % and 29.93±0.48 %, respectively. Also, the AG positive control demonstrated a 64.91±0.89 % inhibitory activity percentage. The observed outcome may plausibly be ascribed to the hindrance of dicarbonyl intermediates from transforming into advanced glycation end products (AGEs). This finding supports the potential of the dietary supplement L-Citrulline to possess encouraging antiglycation characteristics.

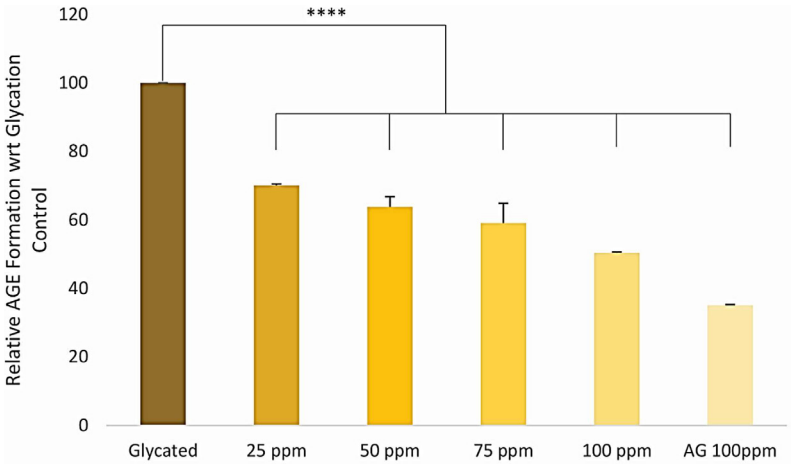

Fig. 2. The Antiglycation (BSA-MGO) effect of the dietary supplement L-Citrulline (25 ppm to 100 ppm) and AG (100 ppm) wrt glycated sample: **** – p<0.0001 wrt control; Dunnett's test; n=3 independent trials

4. 2. Antioxidant Activity of supplement L-Citrulline

Metal Chelating Activity.

The Fe^{2+} ion, classified as a transition metal, can sustain the generation of unpaired electrons, commonly known as free radicals, through electron gain or loss. Consequently, reactive oxygen species formation can be mitigated through metal ion chelation utilizing chelating agents. The chelation capacity of the dietary supplement L-Citrulline was evaluated through a chelation power assay. The reference sample used in this assay was Ascorbic acid. The results demonstrate that the supplement exhibited considerable chelation power. The chelating activities were significant and concentration-dependent, as depicted in Fig. 3.

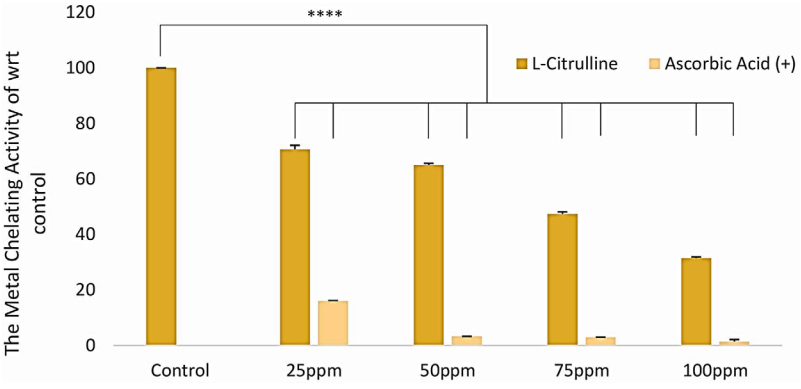

Fig. 3. The Metal Chelating Activity of the dietary supplement L-Citrulline and Ascorbic acid relative to the control: **** – p<0.0001, wrt control; Dunnett's test; n=3 independent trials

The dietary supplement L-Citrulline displayed variable metal chelating activity compared to the control. This activity spanned from 29.33±1.44 % to 68.58±0.45 %. In contrast, Ascorbic acid, another compound studied, showcased a more consistent range of per cent activity differences, ranging from 84.03±0.24 % to 98.54±0.70 %. These results imply that the dietary supplement L-Citrulline exhibits potential in chelating metal ions, which

could have therapeutic implications. Results suggest a promising avenue for further exploration of the metal chelation properties of the supplement.

Scavenging Activity of L-Citrulline towards Glucolipotoxicity-Mediated Reactive Oxygen Species on C2C12 myotubes.

2',7'-Dichlorofluorescin diacetate (DCFDA), a non-fluorescent cell-permeable probe that was deacetylated by cellular esterases to a non-fluorescent compound, which ROS later oxidizes into 2', 7' –dichlorofluorescein (DCF) – a highly fluorescent compound, was used in estimating the level of intracellular reactive species.

Additionally, the DCFDA is not only applicable for the measurement of reactive oxygen species but also in assessing ROS activity mediated by other free radicals or oxidizing reactive nitrogen or carbonyl species. The outcomes of this experiment are depicted in Fig. 4.

Introducing GLT media substantially increased reactive species by approximately 173.48±9.37 % compared to the control group. However, as previously mentioned, adding 10 mM of the supplement showed a significant decrease of 98.42±5.04 % in this escalation. Moreover, when a 10 mM concentration of the dietary supplement L-Citrulline was administered for one hour under standard conditions, it reduced reactive species significantly.

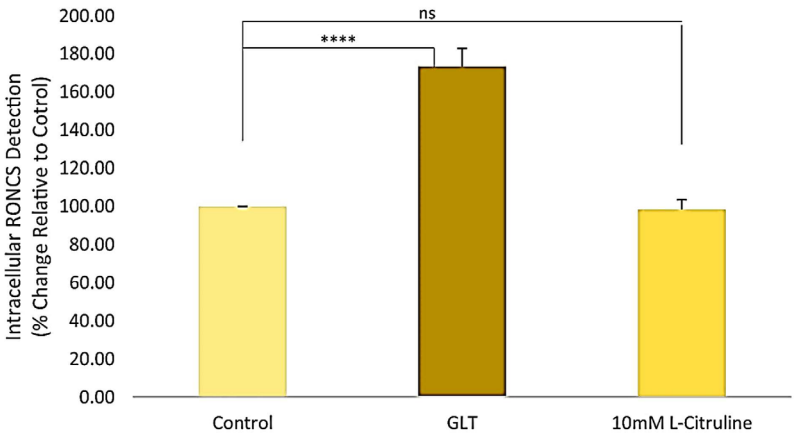

Fig. 4. L-Citrulline effectively scavenges reactive species in C2C12 skeletal muscle cells. Reactive species are expressed as a percentage change in comparison to control: **** – p<0.01 vs Control, ns wrt control; Dunnett's test; n=3 independent trials

These findings highlight the potential of the dietary supplement L-Citrulline to mitigate the increase in reactive species triggered by GLT media inclusion. Hence, the dietary supplement L-Citrulline holds promising therapeutic value in its ability to effectively scavenge reactive oxygen species (ROS) within skeletal muscle cells.

5. Discussion

The process of protein glycation leads to the accumulation of advanced glycation end products (AGEs), which bring about modifications to proteins, resulting in structural and functional impairments, as well as the creation of crosslinks between and within proteins [24]. It is widely accepted that the buildup of crosslinked advanced glycation end products (AGEs) in tissues contributes to the chronic complications associated with diabetes and ageing [25]. The current study investigated the dietary supplement L-Citrulline in vitro antiglycation and antioxidant properties. The study conducted experiments on *in vitro* protein glycation by incubating glucose and methylglyoxal at physiological pH. Bovine serum albumin (BSA) was utilized as a model protein to measure crosslinked AGEs via fluorescence spectrometry. This study employed two distinct BSA models: BSA-glucose and BSA-methylglyoxal (BSA-MGO). Earlier research inquiries have explored the glycation process in both in vitro models [26].

The current study proved that the dietary supplement L-Citrulline displayed strong inhibitory effects on the formation of advanced glycation end products (AGEs) induced by glucose and methylglyoxal in an *in vitro* setting. This inhibition was observed in a dose-dependent manner. The observed ability of the supplement to hinder AGE formation is likely linked to its antioxidant properties. More specifically, the supplement's efficacy lies in its ability to neutralize the radicals produced during glycation. The results suggest that the dietary supplement L-Citrulline operates as an antioxidant, counteracting the activity of free radicals and showing the potential to avert glycation effects. The supplement's capacity to prevent the formation of crosslinks resulting from glycation might be attributed to its potential to bind transition metals, as illuminated by the present investigation. This property could disrupt autoxidative glycation and glycoxidation reactions. Notably, administering L-Citrulline as a supplement was observed to significantly hinder the creation of advanced glycation end products (AGEs). Results imply that the dietary supplement could mitigate the development of AGEs by restraining the reactivity of carbonyl groups within glycated proteins.

The present investigation affirms the inhibitory effect of a supplement on the formation of glycation *in vitro*. Prior research has indicated that the administration of L-citrulline reduced fasting blood glucose (by – 13 %) and glycated haemoglobin (by – 11 %), both of which are recognized indicators of glycemic control compared to a placebo [27] – Additionally, L-citrulline supplementation enhanced glucose uptake by the skeletal muscle [28]. The outcomes of this study indicate that incorporating the dietary supplement L-Citrulline holds promise in reducing the formation of advanced glycation end products (AGEs) by lowering glucose levels. The present investigation's findings highlight that administering the dietary supplement L-Citrulline induces a notable antioxidant impact. The supplement contains molecules containing an amine group that can react with methylglyoxal. This reaction results in the protection of amino groups in proteins from glycation. The supplement may contain additional antioxidant molecules that can protect against autoxidative glycation, ultimately leading to a decrease in the formation of advanced glycation end products (AGEs).

The dietary supplement L-Citrulline also demonstrated antioxidative effects and effectively alleviated oxidative stress. The supplement's potential to impede the creation of advanced glycation end products (AGEs) is likely linked to its ability to chelate metals. Furthermore, it's important to note that the current study did not evaluate oxidative stress markers.

The regular intake of L-Citrulline in the body is approximately 3 grams, derived from dietary sources and endogenous synthesis. However, using L-citrulline as a dietary supplement has gained traction due to its potential to enhance various physiological processes. The results of this study indicate that concentrations ranging from 25 ppm to 100 ppm have shown significant outcomes in terms of antiglycation and antioxidant activities in an *in vitro* context.

At these concentrations, the dietary supplement L-Citrulline exhibits remarkable effects in inhibiting the formation of advanced glycation end products (AGEs) and mitigating oxidative stress. This is crucial because the accumulation of AGEs and oxidative stress are implicated in various health complications, including diabetes and age-related diseases.

Incorporating L-Citrulline through dietary sources or supplements could provide individuals with a means to modulate their antioxidative and antiglycation defences. While there is some indication that L-Citrulline supplementation ingestion, as previously discussed, could potentially reduce hyperglycemia, the direct influence on diminishing advanced glycation end products (AGEs) *in vivo* remains uncertain. Nevertheless, it's vital to consider the utilization of L-Citrulline as an accessible dietary supplement for diabetes management while exploring its potential to provide safeguards against diabetes-related complications against complications that may arise from diabetes.

Research limitations. The *in vitro* examination of the dietary supplement L-Citrulline was conducted. Both cell-free assays and cell-based assays were used in the analysis, with the latter explicitly utilizing only muscle cells from the C2C12 line. The present study conducted several assays in a cell-free environment, including an antiglycation assay using two models, BSA-glucose and BSA-MGO, as well as an antioxidant assay involving iron chelation and a ROS scavenging assay conducted in a cell-based environment.

Prospects for further research. The correlation between antiglycation, antioxidants, and diabetes has been extensively researched, and it has been suggested that L-Citrulline as a dietary supplement, may serve as a foundation for future pharmaceutical development and as a therapeutic agent for the management and treatment of Type 2 Diabetes and its associated complications.

6. Conclusion

Diabetes is a widely recognized global health issue that affects a significant portion of the population. This phenomenon is commonly associated with protein glycation and oxidative stress. The present study evaluates the antiglycation and antioxidant properties current in the dietary supplement L-Citrulline. The potential of supplements to inhibit the formation of advanced glycation end products (AGEs) has been observed through antiglycation assays. At a concentration of 100 ppm, the supplement exhibited a 52.19±0.39 % inhibitory effect on glycation in the BSA-glucose model and a 49.64±0.27 % inhibitory effect in the BSA-MGO model. Additionally, it exhibited a reduced ability of 68.57±0.45 % through iron chelation and demonstrated that the dietary supplement mitigates the production of free radicals and reactive species triggered by glucolipotoxicity.

In light of these findings, the potential of the dietary supplement L-Citrulline as a therapeutic strategy for addressing the glycation response and oxidative stress in individuals with diabetes becomes evident. Compared to other antioxidants studied in the context of diabetes management, the dietary supplement L-Citrulline's unique mechanisms and observed effects stand out.

Traditional antioxidants like vitamin C have demonstrated their ability to counteract oxidative stress by neutralizing free radicals. However, the dietary supplement L-Citrulline offers a distinct approach with its metal-chelating activity and antiglycation properties. While both antioxidants aim to mitigate oxidative damage and glycation, the dietary supplement L-Citrulline's multifaceted attributes present an intriguing alternative or complementary avenue.

The observed inhibitory effects on AGE formation and the ability to mitigate oxidative stress point toward the dietary supplement L-Citrulline's potential efficacy. Compared to established antioxidants, the percentage of inhibitory effects achieved by the dietary supplement L-Citrulline at concentrations ranging from 25 ppm to 100 ppm would offer valuable insight into its relative strength. Additionally, exploring how dietary supplement L-Citrulline's impact translates into tangible clinical outcomes, such as glycemic control and complications prevention, would provide a comprehensive perspective.

This comparison underscores the importance of recognizing that different antioxidants may have varying mechanisms and potency. Dietary supplement L-Citrulline's specific attributes offer a unique angle in the complex landscape of diabetes management, potentially contributing to a more holistic approach. Further research and direct comparative studies between the dietary supplement L-citrulline and other antioxidants will aid in delineating its precise role and value in improving diabetes care.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

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