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Zinc-Cysteine Coupling Demonstrates Potent *In Vitro* Antioxidant Activity and Preserves Cell Viability Under Glucolipotoxicity-Induced Oxidative Stress

Godzelle Ogoc Bulahan[1*], Charlie A. Lavilla Jr.[1]
[1]Department of Chemistry, Mindanao State University-Iligan Institute of Technology, Iligan City, Lanao del Norte, Philippines-9200
[1]godzelle.bulahan0505@g.msuiit.edu.ph, [1]charliejr.lavilla@g.msuiit.edu.ph
*Corresponding Author

***Abstract*—*Type 2 Diabetes (T2D) is characterized by glucolipotoxicity (GLT)-induced oxidative stress, which impairs skeletal muscle cell function by generating excessive reactive species. This study presents the first in vitro evaluation of a zinc-monocysteine complex (ZMC) as a potent antioxidant and cytoprotective agent under GLT-induced metabolic stress. Using C2C12 cells, we demonstrated that neither GLT nor ZMC treatments compromised cell viability, ensuring reliable functional assessments. ZMC exhibited strong radical scavenging activity in DPPH and nitric oxide assays, comparable to ascorbic acid, emphasizing it capacity to neutralize physiologically relevant free radicals implicated in T2D pathology. While cell-based reactive species detection was not conducted, complementary in vitro radical scavenging assays provided critical insights into ZMC's antioxidant potential under GLT-mediated stress. The enhanced antioxidant effect is attributed to the zinc-thiolate coordination, which stabilizes the thiol group and facilitates electron donation, paralleling mechanisms observed in metallothioneins. Overall, this research identifies ZMC as promising candidate to preserve skeletal muscle cell function and integrity under diabetic metabolic conditions. These findings lay foundational groundwork for future in vivo studies and the development of targeted antioxidant therapies for metabolic diseases like T2D.*

***Keywords*—** *Antioxidant, cell viability, glucolipotoxicity, oxidative stress, zinc-monocysteine complex.*

I. Introduction

Type 2 Diabetes (T2D) continues to pose a significant and escalating threat to global health, fueled by intricate metabolic imbalances that disrupt cellular homeostasis. Among these, glucolipotoxicity (GLT)—the chronic elevation of glucose and fatty acids—has emerged as a pivotal driver of oxidative stress (OS), particularly within insulin-sensitive tissues such as skeletal muscle.[1,2] This persistent metabolic stress promotes an overproduction of reactive species (RS), which overwhelm the cell's antioxidant defenses and impair critical functions including insulins signaling and glucose uptake. The resulting oxidative damage undermines cell integrity and viability, exacerbating the progression of metabolic dysfunction.

Zinc, a vital trace mineral, plays multifaceted roles in maintaining cellular redox balance and regulating metabolic pathways. Its therapeutic potential is notably enhanced when chelated to amino acids, improving both bioavailability and intracellular delivery.[3] Among amino acids, L-cysteine stands out due to its thiol (-SH) group, which is a potent nucleophile capable of directly scavenging free radicals and modulating intracellular redox status.[4] The formation of a zinc–monocysteine complex (ZMC) therefore holds promise as a novel antioxidant entity, capable of synergistically combining zinc's redox regulation with cysteine's thiol chemistry.[5]

Despite extensive studies on zinc and L-cysteine individually, the antioxidant and cytoprotective effects of their complex remain unexplored under glucolipotoxicity conditions. This research pioneers the investigation of zinc–L-cysteine complexes *in vitro*, evaluating their radical scavenging capabilities and their ability to maintain skeletal muscle cell viability in the face of GLT-induced oxidative stress. By integrating biochemical assays with cellular models, this study elucidates the mechanisms through which this complex mitigates oxidative damage and supports cellular function. These insights lay a foundation for developing innovative therapeutic approaches targeting oxidative stress in metabolic disorders like T2D.

II. Experimental

Chemicals and Reagents

All chemicals and reagents were reagent-grade. Highly pure ZMC was already synthesized and previously characterized in Niigata University, Japan. C2C12 cells were obtained from ATCC (USA) via ChemoLife Science, with culture reagents and plasticware from Thermo Fisher Scientific through Noveulab and Medtest, Philippines. The majority of other chemicals were obtained via Chemline and Krypton Philippines from Sigma-Aldrich (Singapore), unless otherwise specified.

Cell Culture Assays

The cell culture protocol outlined by Cripps et al. was used to assess ZMC's potential to reduce stress caused by reactive species (RS) in GLT-induced states, with minor modifications.[6]

C2C12 Cell Culture and Treatment

In a humidified atmosphere with 5% CO_2 at 37 °C, C2C12 skeletal myoblasts were kept in high glucose-Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% v/v fetal bovine serum (FBS), 10% v/v heat-inactivated

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newborn calf serum, and 1% v/v penicillin-streptomycin. Once desired confluency was reached, the medium was changed to DMEM supplemented with 2% v/v heat-inactivated horse serum for 7 days to promote myocytic differentiation. Next, the cells were cultured for five more days in ZMC, GLT media (DMEM with 28 mM glucose, 200 µM oleic acid, and 200 µM palmitic acid), and control "healthy" DMEM media (11 mM glucose).

Cell Viability

A calcein AM-based assay was employed to monitor cell viability. Briefly, the C2C12 muscle cells were cultured in healthy DMEM control or GLT-treated media for 5 days before media were aspirated. Subsequently, cells were washed 3 times with Krebs-Ringer buffer (KRB). A final concentration of 5 µM calcein AM cell viability dye (Thermo Fisher Scientific) in KRB was loaded 1 h before media was washed with KRB. Prepared ZMC (15, 30, and 100 µM) solutions were only loaded to GLT-treated media. The cell viability was measured by fluorescence at $\lambda_{excitation}$ and $\lambda_{emission}$ of 490 nm and 520 nm, respectively.

DPPH Radical Scavenging Assay

In this study, the antioxidant potential of ZMC was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, based on the protocol established by Dr. Prieto, with minor modifications.[7] ZMC was dissolved in distilled water at a concentration of 200 and 100 ppm. The reference standard, ascorbic acid (AA), was similarly prepared at similar concentrations, while the DPPH solution was prepared in methanol at a final concentration of 0.2 mM to ensure adequate solubility and radical stability.

Given the photosensitive nature of both DPPH and AA, all solution preparations and assay procedures were carried out in a dark room under red light to minimize degradation and preserve the integrity of the assay. Reaction mixtures were incubated at room temperature, and the absorbance was measured at 517 nm to quantify the degree of radical scavenging. The reduction in absorbance relative to the control was used to evaluate the hydrogen-donating and percent radical scavenging activity (% RSA) of ZMC.

Nitric Oxide Radical Scavenging Assay

To assess nitric oxide (NO) radical scavenging activity, 1.0 mL of 10 mM sodium nitroprusside, 0.25 mL of phosphate-buffered saline (PBS, pH 7.4), and 0.25 mL of the test sample solution—either ZMC or L-cysteine (CYS) at concentrations of 200 ppm or 100 ppm—were added individually into separate test tubes. For two hours, the combinations were kept at 25 °C. After incubation, 1.0 mL of the sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added to 0.5 mL of each reaction mixture, which was then transferred to a new test tube. The mixtures were vortexed and allowed to stand for 5 min to complete the diazotization reaction. Subsequently, 1.0 mL of N-(1-naphthyl)ethylenediamine dihydrochloride (0.1%) was added, mixed thoroughly, and left to stand at room temperature for 30 min. Absorbance was measure at 540 nm using appropriate blank solutions as references. AA served as the positive control. The percentage of NO radical scavenging inhibition was determined and shown as the percent shift in absorbance when compared to the control.

III. Results and Discussion

Cell Viability

To evaluate the impact of treatment conditions on C2C12 cell viability, a calcein AM assay was employed as a critical preliminary step before conducting antioxidant potential analyses. This assay quantifies live cell fluorescence, providing a sensitive measure of viable cell populations. As depicted in Fig. 1, fluorescence intensity remained consistent across all treatment groups, showing no statistically significant differences compared to the healthy control.

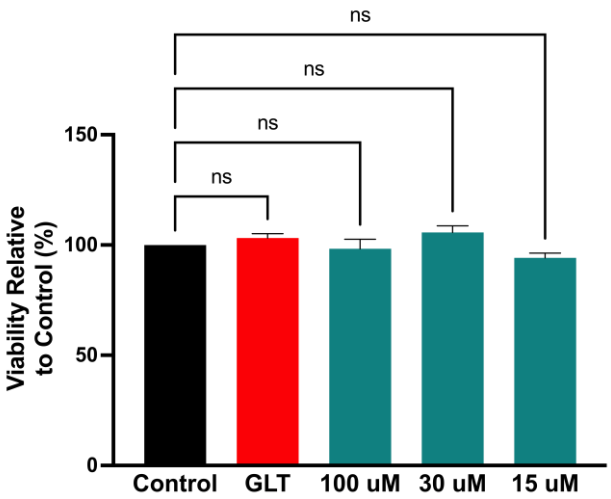

Fig. 1. Cell viability remains unchanged following ZMC (15, 30, and 100 µM) and GLT treatments.

These findings unequivocally demonstrate that neither GLT-mediated stress nor ZMC treatment compromised C2C12 cell viability under the tested conditions. This confirmation is essential, as it validates the experimental model and ensures that subsequent functional assays—specifically RS detection and glucose uptake studies—are not confounded by variations in cell survival or proliferation.

Importantly, the maintenance of cell viability across treatments suggests that the effects attributed to GLT and ZMC are independent of cytotoxicity. Instead, the data imply that ZMC's beneficial influence likely arises from direct biochemical mechanisms, such as mitigating glycation processes, enhancing glucose uptake efficiency, or attenuating oxidative stress. This distinction reinforces the therapeutic potential of zinc–cysteine complexes in protecting muscle cells from metabolic and oxidative insults without inducing cytotoxic effects.

Together, these results provide a robust foundation for interpreting downstream functional outcomes and support further investigation into the molecular pathways modulated by ZMC under GLT-induced stress.

ZMC Antioxidant Activity in DPPH Assay

The antioxidant potential of ZMC was first evaluated using the DPPH radical scavenging assay, a widely accepted method

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for assessing free radical neutralization capacity. As shown in Fig. 2, ZMC exhibited potent radical scavenging activity comparable to the standard antioxidant ascorbic acid (AA) at both 100 ppm and 200 ppm concentrations. Specifically, at 200 ppm, ZMC displayed a 0.2633% higher antioxidant activity relative to CYS, while at 100 ppm, this increase was 0.7338%, as detailed in Table 1. Although these differences did not reach statistical significance ($p > 0.05$), the subtle enhancement in antioxidant capacity may be attributed to the presence of zinc in the complex, a metal known to play critical roles in redox-related biological processes.

TABLE 1. Antioxidant activities of ZMC, CYS, and AA with DPPH.

Sample	Absorbance at 517 nm	% RSA (200, 100 ppm)
Control	1.175	-
ZMC	0.039, 0.039	97.133, 96.903
CYS	0.073, 0.082	96.878, 96.197
AA	0.076, 0.069	97.291, 96.747

Control is DPPH radical absorbance without sample treatment. % RSA was calculated using this equation: [(control-control blank)-(sample-sample blank)]/(control-control blank) x 100%.

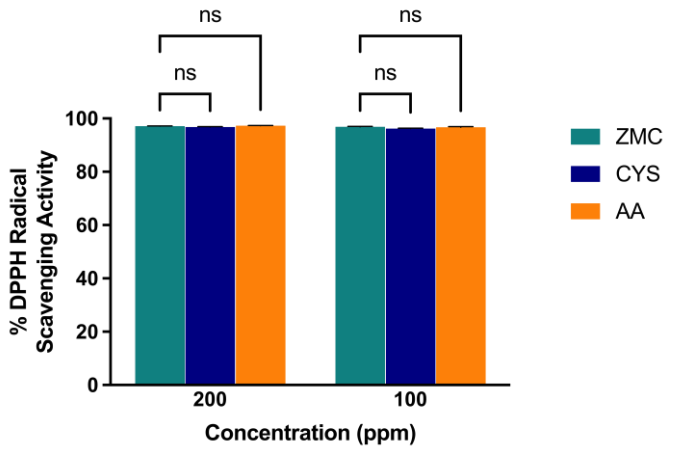


Fig. 2. ZMC is a potent DPPH radical scavenger.

ZMC Nitric Oxide Radical Scavenging Activity

To complement the DPPH findings, the nitric oxide (NO) radical scavenging assay was performed to determine ZMC's efficacy against physiologically relevant NO radicals. Fig. 3 illustrates that ZMC exerted significant NO scavenging activity at both 100 ppm and 200 ppm concentrations. Notably, ZMC showed greater scavenging at 100 ppm than at 200 ppm, suggesting a non-linear, potentially concentration-dependent response. A similar trend was observed with AA, where the lower concentration also resulted in increased NO radical scavenging. However, these differences were not statistically significant ($p > 0.05$), indicating that the observed variations may fall within experimental error margins. Despite this, the consistent pattern across both ZMC and AA may hint at a subtle inverse concentration-response relationship. Contrastingly, CYS demonstrated higher NO scavenging activity at 200 ppm compared to 100 ppm, though at 100 ppm, its activity was lower than that of ZMC. None of these variations achieved statistical significance.

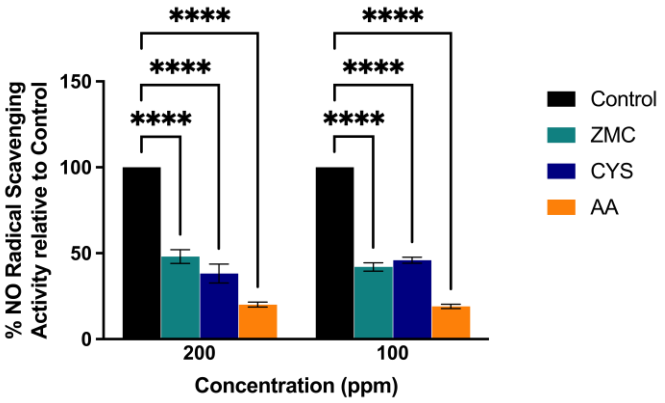


Fig. 3. ZMC demonstrates effective NO radical scavenging power.

Collectively, the DPPH and NO radical scavenging assays confirm that ZMC functions as a robust radical scavenger, underscoring its potential therapeutic utility in mitigating OS-related conditions such as T2D. Preliminary *in vitro* studies further suggest that ZMC may inhibit reactive species generated during GLT-induced OS in C2C12 cells, implicating its role in enhancing glucose uptake in skeletal muscle cells.

The pronounced antioxidant activity of ZMC, as evidenced in Fig. 2 and Fig. 3, can be primarily attributed to the thiolate sulfur coordinated to the zinc ion. This coordination facilitates efficient radical scavenging through electron donation, a mechanism extensively documented for sulfur-containing compounds.[8,9] The Zn-thiolate interaction not only stabilizes the thiolate form but also induces a cyclic conformation that enhances molecular reactivity, consistent with previous studies on zinc-polyphenol and zinc-thiol complexes.[10] Analogous to the redox-active thiolate clusters present in metallothionein proteins, ZMC exhibits a synergistic antioxidant mechanism driven by metal-ligand complexation.[11] While the carboxylate and ammonium groups contribute to aqueous solubility and structural stability, it is the distinctive spatial and electronic configuration conferred by zinc coordination that fundamentally accounts for ZMC's potent free radical scavenging efficacy in both DPPH and NO assays.

IV. Conclusion

This study establishes ZMC as a potent *in vitro* antioxidant with the ability to maintain skeletal muscle cell viability under GLT-mediated metabolic stress. Neither GLT nor ZMC treatments compromised cell viability, validating the model for further functional analyses. ZMC demonstrated robust radical scavenging activity in DPPH and NO assays, underscoring its potential to mitigate OS-associated with T2D. Although this study did not include cell-based reactive species measurements, the strong antioxidant effects observed through chemical assays highlight ZMC's promise in protecting skeletal muscle cells from oxidative damage. The antioxidant efficacy is primarily driven by zinc-thiolate coordination, stabilizing the thiol group and enhancing free radical neutralization. These findings provide a foundation for future research incorporating cellular reactive species detection and *in vivo* models to further elucidate the mechanisms by which

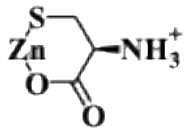
ZMC may improve metabolic function and serve as a therapeutic intervention for oxidative stress-related metabolic diseases.

V. Recommendations

Building on the novel insights gained from this study, future research should extend beyond *in vitro* findings to robust *in vivo* models of type 2 diabetes, enabling a deeper understanding of zinc–L-cysteine complex bioavailability and systemic antioxidant effects. Elucidating the precise molecular pathways by which this complex mitigates oxidative stress and supports insulin signaling will unlock its full therapeutic potential. Additionally, incorporating advanced live-cell reactive species assays will provide richer, dynamic insights into its antioxidant mechanisms within physiological contexts. Efforts to optimize formulation and delivery could amplify its efficacy, paving the way for clinical translation. Ultimately, these steps are essential to transform zinc–L-cysteine complexes from promising biochemical agents into impactful interventions that combat metabolic dysfunction and improve patient outcomes in oxidative stress-driven diseases like diabetes.

Appendix

The figure below presents the proposed structure of the zinc–monocysteine complex investigated in this study, which focuses on evaluating its *in vitro* pharmacological potential.



Appendix 1. Elucidated structure of the zinc-cysteine complex confirmed by various spectroscopic and diffracting techniques.

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References

[1] Zhang, P., Li, T., Wu, X., Nice, E.C., Huang, C., & Zhang, Y. "Oxidative stress and diabetes: antioxidative strategies," *Frontiers in Medicine*, vol. 14, pp. *583-600*, 2020.

[2] Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. "Molecular mechanisms linking oxidative stress and diabetes mellitus," *Oxidative Medicine and Cellular Longevity*, vol. 2020, issue 1, 2020.

[3] Zhang, Y., Li, X., & Liu, X. "A novel zinc complex with antibacterial and antioxidant activity," *BMC Chemistry*, vol 15, article number 17, 2021.

[4] Poole, L. B. "The basics of thiols and cysteines in redox biology and chemistry," *Free Radical Biology and Medicine*, vol. 80, pp. 148-157, 2015.

[5] Ramorobi, L. M., Matowane, G. R., Mashele, S. S., Swain, S. S., Makhafola, T. J., Mfengwana, P. H., & Chukwuma C. I. "Zinc (II)-syringic acid complexation synergistically exerts antioxidant action and modulates glucose uptake and utilization in L-6 myotubes and rat muscle tissue," *Biomedicine & Pharmacotherapy*, vol. 154, 2022.

[6] Cripps, M. J., Hanna, K., Lavilla Jr., C., Sayers, S. R., Caton, P. W., Sims, C., Girolamo, L. D., Sale, C., & Turner, M. D. "Carnosine scavenging of glucolipotoxic free radicals enhances insulin secretion and glucose uptake," *Scientific Reports*, vol. 7, issue 1, pp. 1-7, 2017.

[7] Prieto, J. "Procedure: preparation of dpph radical, and antioxidant scavenging assay," pp. 7-9, 2012, unpublished.

[8] Noguchi, N., Saito, Y., & Niki, E. "Actions of thiols, persulfides, polysulfides as free radical scavenging antioxidants," *Antioxidants and Redox Signaling*, vol. 39, issue 10-12, pp. 728-743, 2023.

[9] Kim, J. H., Jang, H. J., Cho, W. Y., Yeon, S. J., & Lee, C. H. "In vitro antioxidant actions of sulfur-containing amino acids," *Arabian Journal of Chemistry*, vol. 13, issue 1, pp. 1678-1684, 2020.

[10] Zhao, J., Qian, D., Zhang, L., Wang, X., Zhang, J. "Improved antioxidative and antibacterial activity of epigallocatechin gallate derivative complexed by zinc cations and chitosan," *The Royal Society of Chemistry Advances*, vol.14, issue 15, pp. 10410-10415, 2024.

[11] Maret, W., Vallee, B. L. "Thiolate ligands in metallothionein conder redox activity on zinc clusters," *Proceedings of the National Academy of the Unites States od America*, vol. 95, issue 7, pp. 3478-3482, 1998.