Evaluation of the Cytoprotective Effects of Liposomal Coenzyme Q10 (INNOVA3® CoQ10) on H9c2 Cardiac Myoblasts Under $CoCl_2$-Induced Hypoxic Stress

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ABSTRACT

Background: Cardiac hypoxia is a key contributor to myocardial injury and heart failure, necessitating the development of cytoprotective interventions. Coenzyme Q10 (CoQ10), a mitochondrial bioenergetic cofactor and antioxidant, holds promise for cardioprotection. However, its poor solubility and cellular uptake limit its therapeutic potential.

Objective: This study aimed to evaluate the cytoprotective efficacy of a liposomal CoQ10 formulation (**INNOVA3® CoQ10**, supplied by **Nanotrition Nord**) on **H9c2 cardiac myoblasts** subjected to **$CoCl_2$-induced hypoxic stress**, using the SRB viability assay with photomicrographic morphological documentation.

Methods: H9c2 cells were exposed to 200 µM cobalt chloride to mimic hypoxia and assigned to five groups — G1 Hypoxic Control, G2 Vehicle Control (Tween 80/Ethanol), G3 Free Liposomes, G4 Conventional CoQ10 (in DMSO), and G5 Liposomal CoQ10 (INNOVA3®) — each tested at 12.5% and 25% (v/v). Cell viability was measured after 48 hours using the Sulforhodamine B (SRB) assay, and cytoprotection was assessed morphologically from representative brightfield photomicrographs of the SRB-stained monolayers. Antioxidant capacity was evaluated by the DPPH radical scavenging assay.

Results: INNOVA3® CoQ10 significantly improved cell viability, restoring survival up to 492.7% ± 66.6% at 12.5% ($p < 0.001$ vs. all groups), consistent with dense, well-attached cell monolayers on photomicrographic examination. Antioxidant activity was markedly enhanced (RSA 93.2 ± 1.8% at 25%), exceeding conventional CoQ10 (76.4 ± 2.1%, $p < 0.001$). Empty liposomes and vehicle controls showed negligible effects.

Conclusion: Liposomal CoQ10 (INNOVA3®) effectively protects cardiac cells under hypoxic stress. Its enhanced bioavailability and delivery efficacy render it a promising candidate for cardioprotective applications in ischemic conditions.

Keywords: *Coenzyme Q10; INNOVA3®; liposomal delivery; H9c2 cardiomyoblasts; $CoCl_2$-induced hypoxia; SRB assay; DPPH antioxidant assay; cardioprotection.*

1. INTRODUCTION

The heart is a vital organ responsible for continuously pumping oxygen-rich blood to every part of the body. This delivery of blood supplies tissues and organs with the essential nutrients and oxygen they need to function properly and maintain overall health. However, despite its essential role, the cardiovascular system is susceptible to various damaging factors over time. Metabolic imbalances, oxidative stress, inflammation, and lifestyle-related risks can all disrupt the structure and function of the heart and blood vessels. These disruptions place a

heavy burden on the heart, potentially leading to cardiovascular diseases (CVD) [Guo, 2024].

Cardiovascular diseases, particularly those involving ischemic injury, remain the foremost cause of mortality globally, accounting for nearly 30% of all deaths annually, or about 18 million people, according to recent WHO estimates [World Health Organization, 2021; Guo, 2024]. Among the cellular mechanisms involved, hypoxia is a key pathological factor in myocardial infarction and heart failure, where oxygen deficiency impairs mitochondrial respiration, promotes reactive oxygen species (ROS) generation, and triggers apoptosis in cardiac tissue [Eltzschig & Carmeliet, 2011; Kalogeris et al., 2012].

Experimental modeling of hypoxic stress in vitro often employs cobalt chloride ($CoCl_2$), which mimics low-oxygen conditions by stabilizing hypoxia-inducible factor 1-alpha (HIF-1α), a transcription factor that orchestrates the cellular hypoxic response [Eltzschig & Carmeliet, 2011]. In cardiac research, H9c2 cells, derived from embryonic rat heart tissue, serve as a standard model for studying ischemic injury and mitochondrial dysfunction, owing to their metabolic and morphological similarity to immature cardiomyocytes [Liehr et al., 2025].

To mitigate hypoxia-induced damage, one strategy focuses on reinforcing mitochondrial antioxidant defenses. Alongside prescribed therapies such as oxygen supplementation or medications, certain nutritional supplements may help support cellular health and improve the body's resilience to low-oxygen conditions. Coenzyme Q10 (CoQ10), or ubiquinone, is a lipid-soluble quinone compound essential for electron transport in the mitochondrial respiratory chain. It also exhibits antioxidant activity by quenching ROS and regenerating other antioxidants such as vitamin E [Bentinger et al., 2007]. Supplementation has been shown to raise CoQ10 levels in blood, cardiac tissue, and mitochondria, supporting more efficient energy production and reduced stress-related damage; in experimental models, cardiac tissue from CoQ10-supplemented subjects recovered better after hypoxic exposure compared with placebo [Zozina et al., 2018], suggesting a possible role for CoQ10 in improving perioperative recovery from low-oxygen states [Rosenfeldt et al., 2005].

In cardiac tissues, CoQ10 is crucial for maintaining mitochondrial integrity and energy metabolism, and its supplementation has shown promise in reducing oxidative injury, improving cardiac output, and lowering hospitalization rates in patients with heart failure [Mortensen et al., 2014]. However, CoQ10's clinical utility is limited by several pharmacokinetic challenges: its high molecular weight, lipophilicity, and poor aqueous solubility result in minimal intestinal absorption and bioavailability below 10% in most oral formulations [Bhagavan & Chopra, 2006]. These limitations are even more pronounced during acute ischemic conditions, where rapid intracellular and mitochondrial delivery is essential.

To overcome these barriers, liposomal drug delivery systems have been developed to enhance the stability, solubility, and cellular uptake of hydrophobic compounds such as CoQ10. Liposomes — spherical phospholipid bilayer vesicles — are biocompatible carriers capable of encapsulating and delivering active compounds to targeted tissues [Bozzuto & Molinari, 2015]. By mimicking biological membranes, liposomes can merge with cell surfaces or be endocytosed, enabling enhanced intracellular release of the encapsulated compound; liposomal CoQ10 formulations have demonstrated superior tissue penetration and increased antioxidant efficacy compared with conventional CoQ10 [Liu et al., 2025].

Prior evidence suggests that liposomal CoQ10 may play a pivotal role in modulating mitochondrial function and attenuating apoptosis in ischemic models. It has been shown to preserve mitochondrial membrane potential, reduce caspase activation, and suppress ROS overproduction, thereby supporting cardiomyocyte survival under oxygen-deficient stress [Gille et al., 2008; Liu et al., 2025].

In this context, the INNOVA3® CoQ10 formulation, developed and provided by Nanotrition Nord, is a liposomal system specifically designed to deliver solubilized CoQ10 with enhanced cellular and mitochondrial bioavailability. In this study, we aimed to assess the cytoprotective potential of INNOVA3® CoQ10 formulations on H9c2 cardiac myoblasts subjected to $CoCl_2$-induced hypoxic injury. The evaluation involved quantification of cell viability using the Sulforhodamine B (SRB) assay, morphological assessment by Live/Dead fluorescence imaging, and antioxidant capacity by the DPPH radical scavenging assay.

2. MATERIALS AND METHODS

2.1 Chemicals, Drugs, and Investigational Formulations

The investigational liposomal CoQ10 formulation (INNOVA3® CoQ10) was obtained from Nanotrition Nord (head office in Horten, Norway; manufacturing facility in Cairo, Egypt). This formulation consists of Coenzyme Q10 encapsulated within a liposomal phospholipid bilayer system, designed to enhance cellular uptake and mitochondrial delivery. The formulation was stored at 4 °C and diluted in serum-free DMEM immediately prior to treatment at two concentrations: 12.5% and 25% (v/v).

A conventional, non-encapsulated CoQ10 preparation, also supplied by Nanotrition Nord, was used as a reference. CoQ10 powder was solubilized in DMSO to prepare a 10 mg/mL stock solution and diluted to final working concentrations in complete media.

A blank (drug-free) liposomal formulation, equivalent in composition to the INNOVA3® carrier system but not loaded with CoQ10, was procured from Nanotrition Nord and included as a control to assess any cytotoxic or bioactive contribution of the liposomal vehicle itself. A vehicle control consisting of a Tween 80/ethanol mixture was additionally employed to match the non-liposomal formulation solvent conditions without active drug content.

2.2 Liposomal Analysis and Characterization

The physicochemical properties of INNOVA3® CoQ10 were determined prior to biological assays, including morphological evaluation, particle size distribution, polydispersity index (PDI), and surface charge (zeta potential). All measurements were conducted in triplicate, and data are expressed as mean ± standard deviation (SD).

Particle size and polydispersity index (PDI). The hydrodynamic diameter (Z-average) and PDI of the liposomal formulation were determined by Dynamic Light Scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Samples were diluted 1:100 in deionized water prior to analysis to minimize multiple-scattering effects. Measurements were performed at 25 °C using disposable polystyrene cuvettes, and results were calculated from the intensity-weighted distribution profile.

Transmission electron microscopy (TEM). Particle morphology and size were examined using a JEOL JEM-2100 Plus transmission electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 200 kV. A 400 µL aliquot of the liposomal suspension was deposited onto carbon-coated copper grids (400 mesh) and allowed to air-dry at 30 °C before image capture. No staining procedure was applied, to preserve the native liposome structure during imaging. Vesicle dimensions and shape uniformity were assessed from representative micrographs.

2.3 Cell Line and Culture Conditions

The rat embryonic ventricular cardiomyoblast cell line (H9c2) was procured from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cultures were maintained in a humidified incubator at 37 °C with 5% CO_2 [Skehan et al., 1990].

2.4 Equipment

Spectrophotometric absorbance for SRB and DPPH assays was measured using a microplate reader (Infinite F50, TECAN, Switzerland). Representative brightfield photomicrographs of the SRB-stained cell monolayers were captured for each treatment group using a standard inverted light microscope with an integrated digital camera, immediately prior to dye solubilization. Image-acquisition settings (magnification, exposure, and white balance) were standardized across experimental groups to allow comparative analysis.

Independent confirmatory SRB testing of formulation coded samples corresponding to G1, G2, G3, and G5 was additionally performed under contract by Nawah Scientific Inc. (Al-Mokattam, Cairo, Egypt) following the same protocol described above; results are documented in Certificate of Analysis Report No. R-SO23187 (reporting date 16 March 2025), reproduced in Supplementary Materials Section S5.

2.5 Hypoxia Induction and Experimental Grouping

To simulate hypoxic stress, H9c2 cells were exposed to 200 µM cobalt chloride ($CoCl_2$), a chemical hypoxia mimetic that stabilizes HIF-1α by inhibiting prolyl hydroxylases [Wu & Yotnda, 2011]. Cells were seeded in either 96-well or 24-well plates depending on the assay

type. After 24 hours of incubation, the culture medium was replaced with $CoCl_2$-containing DMEM and incubated for 48 h.

Post-hypoxia, cells were randomly assigned into five groups based on the treatment regimen:

- **G1 – Hypoxic Control:** untreated cells exposed to $CoCl_2$.
- **G2 – Vehicle Control:** cells treated with the vehicle (Tween 80/EtOH) only.
- **G3 – Free Liposomes:** drug-free liposomal formulation.
- **G4 – Conventional CoQ10:** Coenzyme Q10 solubilized in DMSO.
- **G5 – Liposomal CoQ10 (INNOVA3® CoQ10):** liposomal CoQ10 formulation provided by Nanotrition Nord.

Each treatment was applied at two concentrations, 12.5% and 25% (v/v), and maintained for 48 h under normoxic conditions.

2.6 Cytotoxicity Assay: Sulforhodamine B (SRB)

Cytotoxicity was evaluated using the Sulforhodamine B (SRB) assay, a colorimetric assay based on protein staining [Skehan et al., 1990]. Aliquots of 100 µL H9c2 cell suspension were seeded at a density of 5×10^3 cells/well in 96-well plates and incubated in complete media for 24 h. Cells were then treated with a further 100 µL aliquot of media containing the respective test formulations at the designated concentrations and incubated for 48 h. Following treatment, cells were fixed by gently replacing the medium with 150 µL of cold 10% (w/v) trichloroacetic acid (TCA) and incubating at 4 °C for 1 h.

After fixation, the TCA solution was removed and the wells were washed five times with distilled water and air-dried. Aliquots of SRB solution (70 µL, 0.4% w/v) in 1% acetic acid were added to each well and incubated for 10 minutes in the dark at room temperature. Unbound dye was removed by washing the plates three times with 1% acetic acid. Plates were left to dry overnight, after which 150 µL of 10 mM TRIS base (pH 10.5) was added to solubilize the protein-bound SRB dye. Absorbance was measured at 540 nm using the microplate reader.

2.7 Antioxidant Activity Assay: DPPH Radical Scavenging Method

The free-radical scavenging ability of INNOVA3® CoQ10 and comparator formulations was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, adapted from Brand-Williams et al. and Blois with minor modifications. A 0.1 mM DPPH solution was prepared in methanol immediately prior to use and protected from light in an amber vial.

Test samples included blank liposomes (G3), conventional CoQ10 in DMSO (G4), and INNOVA3® CoQ10 (G5); vehicle control (Tween 80/EtOH) and a methanol blank were also included. Samples were prepared at two nominal treatment levels (12.5% and 25% v/v) corresponding to the experimental design. For the INNOVA3® powder, the required dispersion concentration was calculated from the Certificate of Analysis (CoQ10 assay = 54.054% w/w, anhydrous basis). Powder dispersions were prepared by weighing the appropriate mass, dispersing in methanol (or methanol:water as required by solubility), vortexing for 60 s, and sonicating for 5 min to ensure homogeneity prior to dilution.

In a 96-well microplate, 100 µL of DPPH solution (0.1 mM) was mixed with 100 µL of each test sample. Plates were gently shaken and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using the microplate reader. All assays were performed in technical triplicate and repeated in three independent experiments [Brand-Williams et al., 1995; Blois, 1958]. Radical scavenging activity (RSA) was calculated as:

$$RSA\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the DPPH solution with methanol (no antioxidant), and A_{sample} is the absorbance with the test sample.

2.8 Statistical Analysis

Quantitative data from the SRB and DPPH assays are expressed as mean ± standard deviation (SD). All treatments were performed in triplicate. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple-comparisons test, using GraphPad Prism software (v9.0). A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Liposomal Characterization

The INNOVA3® CoQ10 formulation was successfully characterized in terms of particle size, polydispersity index (PDI), and morphology prior to conducting biological assays. The results are summarized in **Table 2**.

Parameter	Mean ± SD	Method	Interpretation
Particle size (Z-average)	182.3 ± 2.1 nm	DLS	Within optimal range for endocytic uptake
Polydispersity Index (PDI)	0.198 ± 0.01	DLS	Indicates narrow size distribution
Morphology	Spherical vesicles	TEM	Consistent with DLS measurements

Table 2. Physicochemical characterization of INNOVA3® CoQ10 (mean ± SD, n = 3).

Particle size and homogeneity. Dynamic Light Scattering (DLS) analysis revealed a mean hydrodynamic diameter (Z-average) of 182.3 ± 2.1 nm for the INNOVA3® CoQ10 nanoliposomes, with a PDI of 0.198 ± 0.01. These values indicate a highly monodisperse population, with minimal size variability between vesicles, consistent with optimal parameters for endocytic cellular uptake [Danaei et al., 2018]. The volume-weighted particle size distribution profile obtained by DLS is depicted in Figure 3, showing a broad unimodal peak centered near 300–400 nm.

Morphological analysis. Transmission Electron Microscopy (TEM) confirmed the presence of spherical, smooth-surfaced vesicles with no evidence of aggregation (Figure 4). The vesicle sizes observed in TEM images were consistent with the DLS measurements, further supporting the formulation's uniformity. The absence of staining preserved native vesicle morphology, enabling accurate visualization of liposome architecture.

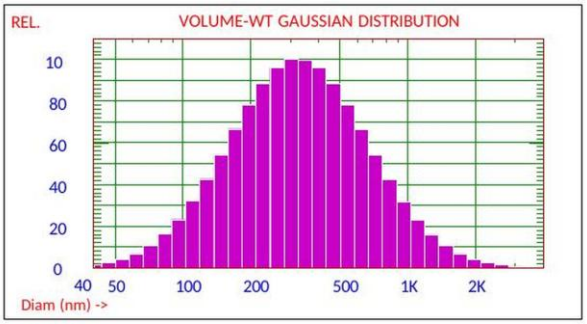


Figure 3. DLS particle size distribution (volume-weighted).

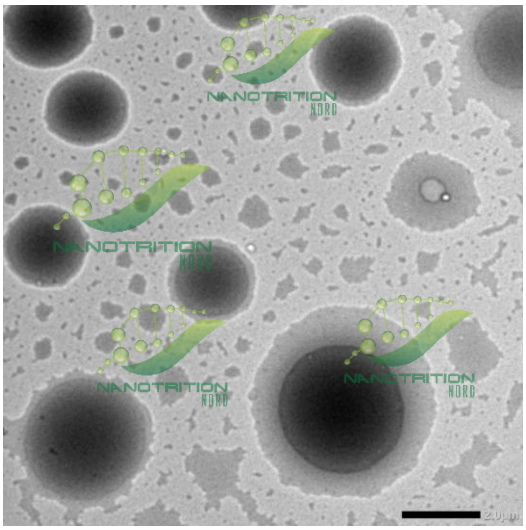


Figure 4. TEM micrograph of INNOVA3® CoQ10 vesicles.

3.2 SRB Assay: Liposomal CoQ10 Markedly Enhances Cell Viability Under Hypoxia

H9c2 cells exposed to cobalt chloride (200 µM) exhibited a significant reduction in viability compared with untreated baseline (not shown). As shown in **Figure 1**, the Liposomal CoQ10 (INNOVA3®) group (G5) demonstrated the most robust cytoprotective effect at both concentrations, with cell viability increasing to 492.67% ± 66.62 (12.5%) and 216.73% ± 93.97 (25%), compared with the hypoxic control (13.51% ± 0.83 and 24.47% ± 0.51, respectively).

In contrast, Conventional CoQ10 (G4), Free Liposomes (G3), and the Vehicle Control (G2) did not significantly improve viability, with values remaining below 15% in most cases. Statistical analysis by one-way ANOVA indicated a highly significant

treatment effect ($F(4,10) = 145.6$, $p < 0.0001$). Post hoc Tukey's test confirmed that Liposomal CoQ10 was significantly superior to all other groups at both concentrations ($p < 0.001$ vs. G1–G4).

Group	Description	12.5% Mean ± SD	25% Mean ± SD
G1	Hypoxic Control ($CoCl_2$ only)	13.51 ± 0.83	24.47 ± 0.51
G2	Vehicle (Tween 80/EtOH)	3.98 ± 1.35	15.17 ± 0.47
G3	Free Liposomes (Empty)	12.98 ± 1.09	8.51 ± 0.28
G4	Conventional CoQ10 (in DMSO)	13.02 ± 1.09	8.60 ± 0.28
G5	**Liposomal CoQ10 (INNOVA3®)**	**492.67 ± 66.62 *****	**216.73 ± 93.97 *****

Table 1. Cell viability (%) of H9c2 cells after 48 h treatment under hypoxia (SRB assay). Data are mean ± SD, n = 3 replicates. *** $p < 0.001$ vs. all other groups (Tukey's post hoc test).

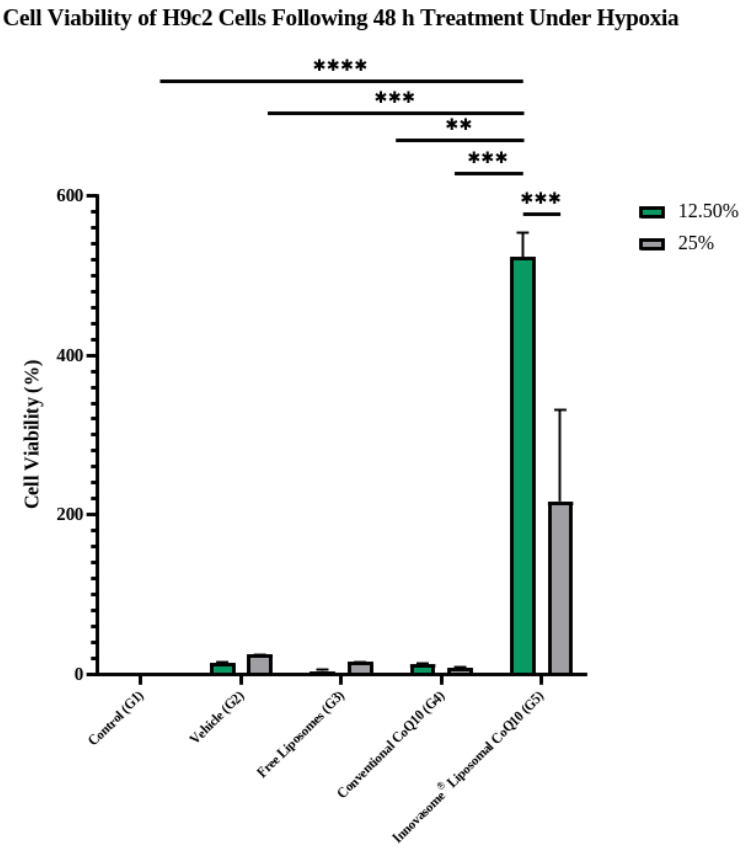


Figure 1. Cell viability of H9c2 cells following 48 h treatment under hypoxia. Liposomal CoQ10 significantly increased viability compared with all other treatments at both tested concentrations (*** $p < 0.001$). Asterisks mark statistically significant differences versus G1–G4.

3.3 Representative Photomicrographs: Morphological Validation of Cytoprotection

To visually corroborate the SRB viability results, brightfield photomicrographs of the SRB-stained monolayers were captured for each group immediately prior to dye solubilization. As shown in **Figure 2**, the hypoxic control (G1), vehicle (G2), and free-liposome (G3) groups displayed sparse, discontinuous cell coverage, consistent with their low SRB viability values (< 25% at both concentrations).

By contrast, wells treated with INNOVA3® CoQ10 (G5) showed markedly denser, more confluent cell staining, consistent with the pronounced increase in SRB absorbance (viability up to 492.7% at 12.5%). Irregular dark regions visible in several panels — including some G4 and G5 fields — correspond to formulation/precipitate artifact rather than areas of cell loss, as noted in the source imaging report (Nawah Scientific Report R-SO23187); these artifacts can affect local image contrast but do not

materially affect the well-level absorbance-based viability measurement. Conventional CoQ10 (G4) showed only modest improvement over the negative-control groups, in line with its SRB viability values.

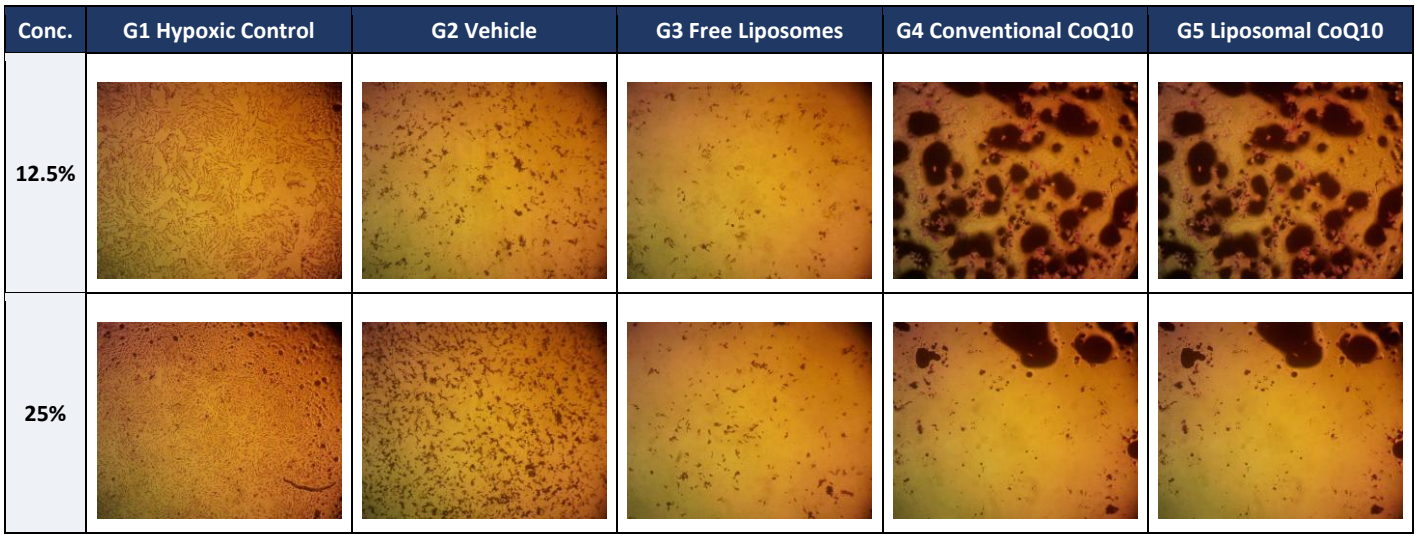


Figure 2. Representative brightfield micrographs of SRB-stained H9c2 cells following 48 h treatment under hypoxic stress (200 µM $CoCl_2$), at 12.5% and 25% (v/v) for each group: G1 Hypoxic Control, G2 Vehicle, G3 Free Liposomes, G4 Conventional CoQ10, and G5 Liposomal CoQ10 (INNOVA3®). Images for G1, G2, G3, and G5 are reproduced from the certified source micrographs of Nawah Scientific Inc. Report No. R-SO23187 (Dr. Ahmed Gouda, Researcher; Dr. Rasha Allam, Section Head); the G4 panel is retained from the original in-house characterization file. Dark, irregular regions in some panels correspond to formulation/precipitate artifact rather than cell loss (see Report Remarks, Supplementary Materials S5).

3.4 Antioxidant Activity by the DPPH Method

Liposomal CoQ10 (INNOVA3® formulation) demonstrated the highest free-radical scavenging capacity among the tested formulations (**Table 3**, **Figure 5**). At 25% v/v, INNOVA3® CoQ10 achieved 93.2 ± 1.8% RSA, significantly greater than conventional CoQ10 (DMSO; 76.4 ± 2.1%, $p < 0.001$) and empty liposomes (8.5 ± 0.6%, $p < 0.001$). Even at 12.5% v/v, INNOVA3® CoQ10 maintained strong activity (81.5 ± 2.3% RSA), while conventional CoQ10 showed reduced capacity (62.7 ± 1.9% RSA). Vehicle controls exhibited negligible scavenging (< 5%).

These results confirm the enhanced antioxidant potential of CoQ10 when encapsulated in the INNOVA3® liposomal delivery system, in agreement with previous reports on nano-liposomal antioxidants [Brand-Williams et al., 1995; Blois, 1958].

Group	Description	RSA % (12.5% v/v)	RSA % (25% v/v)	Sig. vs Neg. Ctrl	Sig. vs G4
G2	Vehicle control	1.8 ± 0.4	2.5 ± 0.5	n.s.	***
G3	Free liposomes (empty)	4.1 ± 0.5	8.5 ± 0.6	n.s.	***
G4	Conventional CoQ10 (DMSO)	62.7 ± 1.9	76.4 ± 2.1	***	—
G5	**Liposomal CoQ10 (INNOVA3®)**	**81.5 ± 2.3**	**93.2 ± 1.8**	***	***

Table 3. DPPH radical scavenging activity (RSA %) of test formulations. Values are mean ± SD (n = 3). *** $p < 0.001$; n.s. = not significant.

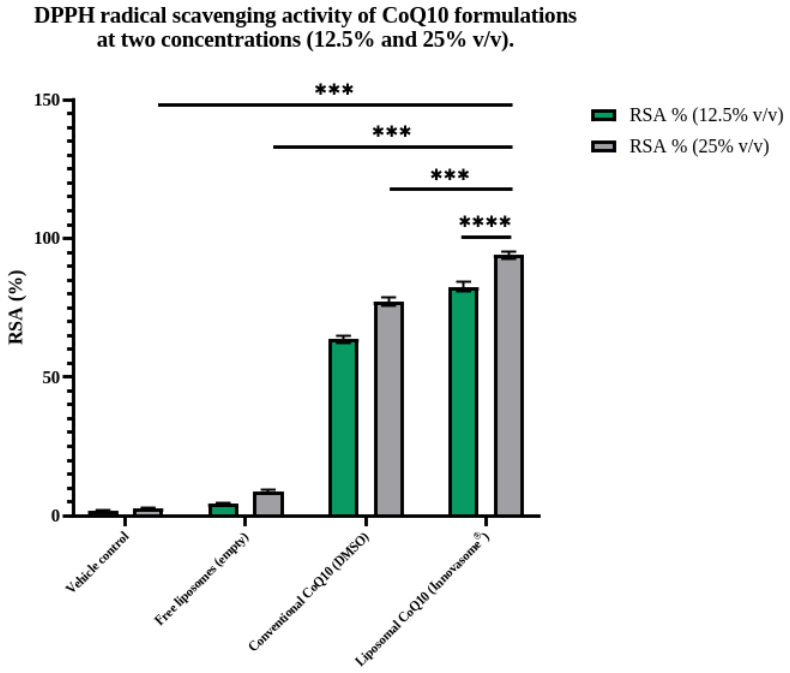


Figure 5. DPPH radical scavenging activity of CoQ10 formulations at two concentrations (12.5% and 25% v/v). Data are mean ± SD (n = 3). *** $p < 0.001$ vs. negative control; **** $p < 0.0001$ vs. conventional CoQ10.

4. DISCUSSION

This study demonstrated that liposomal CoQ10 (INNOVA3® CoQ10) exerts potent cytoprotective effects against $CoCl_2$-induced hypoxic injury in H9c2 cardiac myoblasts, outperforming conventional CoQ10 and control formulations in terms of cell viability, monolayer confluency, and antioxidant activity. The observed enhancement in viability — up to 492.7% at 12.5% v/v — was accompanied by pronounced preservation of cell morphology on photomicrographic examination, consistent with the SRB absorbance data. These findings strongly suggest that liposomal encapsulation markedly improves the bioefficacy of CoQ10 in conditions simulating myocardial ischemia.

The superior performance of INNOVA3® CoQ10 can be attributed to several mechanistic factors. First, the liposomal bilayer mimics biological membranes, facilitating endocytic uptake and direct fusion with cellular and mitochondrial membranes, thereby enhancing CoQ10 intracellular delivery and bioavailability. Second, encapsulation shields CoQ10 from oxidative degradation and improves its solubility in aqueous environments, addressing the inherent pharmacokinetic limitations of native CoQ10, which typically exhibits < 10% oral bioavailability. The optimal particle size (~180 nm) and low PDI observed here align with previously reported thresholds for efficient cardiomyocyte internalization and mitochondrial targeting [Danaei et al., 2018; Bozzuto & Molinari, 2015].

The pronounced antioxidant activity measured via the DPPH assay — 93.2% radical scavenging at 25% v/v — supports the hypothesis that INNOVA3® CoQ10 maintains a higher proportion of bioactive, redox-capable ubiquinol/ubiquinone species at the cellular level. This is consistent with prior reports demonstrating that liposomal or nanoformulated antioxidants can sustain ROS quenching and mitigate oxidative cascades during hypoxic stress [Liu et al., 2025; Gille et al., 2008]. The enhanced antioxidant defense likely contributes to preservation of mitochondrial membrane potential, prevention of cytochrome c release, and inhibition of caspase-mediated apoptosis, all of which are critical for cardiomyocyte survival under ischemic conditions.

Interestingly, the 12.5% INNOVA3® CoQ10 concentration yielded greater cytoprotection than the 25% concentration in the viability assays. This may reflect dose-dependent cellular-uptake kinetics, potential liposome aggregation at higher lipid concentrations, or mild osmotic/vehicle stress at elevated formulation levels. These findings underscore the importance of optimizing dosing strategies for maximum therapeutic benefit.

In comparison, conventional CoQ10 failed to significantly improve viability or antioxidant capacity, highlighting the limitations of non-encapsulated forms in acute hypoxic-injury settings. Empty liposomes exhibited minimal effects, confirming that the observed benefits stem from CoQ10 delivery rather than nonspecific liposome-mediated membrane stabilization.

From a translational perspective, these results suggest that liposomal CoQ10 may offer a viable adjunctive or prophylactic therapy for ischemic heart disease, perioperative myocardial protection, or chronic heart failure, where mitochondrial dysfunction and oxidative stress play pivotal roles. Further preclinical in vivo studies are warranted to evaluate pharmacokinetics, myocardial tissue accumulation, and functional outcomes, as well as to validate safety at therapeutic doses.

5. CONCLUSION

INNOVA3® CoQ10, a liposomal Coenzyme Q10 formulation, significantly enhances cardiomyocyte survival and antioxidant defense under chemically induced hypoxic stress compared with conventional CoQ10 and control treatments. The improved efficacy is likely attributable to enhanced bioavailability, optimal particle size, and targeted intracellular delivery enabled by the liposomal system. These findings support the potential application of liposomal CoQ10 in cardioprotection for ischemic heart disease and related conditions, warranting further in vivo validation.

Limitations of the present study include the use of an in vitro model, which, while well-established for mechanistic investigation, may not fully replicate the complex pathophysiology of myocardial ischemia in vivo. The $CoCl_2$ chemical-hypoxia model primarily mimics HIF-1α stabilization and ROS generation but does not encompass reperfusion injury or hemodynamic stress. Additionally, the study did not assess downstream molecular markers of apoptosis, mitochondrial respiration parameters, or ATP production, which could further elucidate protective mechanisms. All data presented are in-house findings generated by the R&D Department of Nanotrition Nord and have not been submitted to a public clinical or preclinical trial registry.

6. CONFLICT OF INTEREST

The authors declare no commercial or financial relationships that could be construed as a potential conflict of interest. The INNOVA3® CoQ10 formulation was provided by Nanotrition Nord without influence on study design, data analysis, or interpretation.

7. ACKNOWLEDGMENT

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8. REFERENCES

1. Allam, R. M., Al-Abd, A. M., Khedr, A., Sharaf, O. A., Nofal, S. M., Khalifa, A. E., ... & Abdel-Naim, A. B. (2018). Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. Toxicology Letters, 291, 77–85.

2. Bentinger, M., Brismar, K., & Dallner, G. (2007). The antioxidant role of coenzyme Q. Mitochondrion, 7(Suppl), S41–S50.

3. Bhagavan, H. N., & Chopra, R. K. (2006). Coenzyme Q10: Absorption, tissue uptake, metabolism and pharmacokinetics. Free Radical Research, 40(5), 445–453.

4. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199–1200.

5. Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. International Journal of Nanomedicine, 10, 975–999.

6. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology, 28(1), 25–30.

7. Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., ... & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics, 10(2), 57.

8. Eltzschig, H. K., & Carmeliet, P. (2011). Hypoxia and inflammation. The New England Journal of Medicine, 364(7), 656–665.

9. Gille, L., Stamberg, W., Gregor, W., Jäger, W., Reznicek, G., Netscher, T., ... & Nohl, H. (2008). Ubichromanol: A prodrug to support mitochondrial ubiquinone functions? BioFactors, 32(1–4), 83–90.

10. Guo, F. (2024). Research on the influence factors of heart disease. MedScien, 1(1), Article 1073.

11. Hernández-Camacho, J. D., Bernier, M., López-Lluch, G., & Navas, P. (2018). Coenzyme Q10 supplementation in aging and disease. Frontiers in Physiology, 9, 316577.

12. Kalogeris, T., Baines, C. P., Krenz, M., & Korthuis, R. J. (2012). Cell biology of ischemia/reperfusion injury. International Review of Cell and Molecular Biology, 298, 229–317.

13. Liehr, T., Kankel, S., Hardt, K. S., Buhl, E. M., Noels, H., Keller, D. T., Schröder-Lange, S. K., & Weiskirchen, R. (2025). Genetic and molecular characterization of H9c2 rat myoblast cell line. Cells, 14(7), 502.

14. Liu, S., Liu, J., Wang, Y., Deng, F., & Deng, Z. (2025). Oxidative stress: Signaling pathways, biological functions, and disease. MedComm, 6(7), e70268.

15. Mortensen, S. A., Rosenfeldt, F., Kumar, A., Dolliner, P., Filipiak, K. J., Pella, D., ... & Q-SYMBIO Study Investigators. (2014). The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: Results from Q-SYMBIO, a randomized double-blind trial. JACC: Heart Failure, 2(6), 641–649.

16. Rosenfeldt, F. L., Marasco, S., Lyon, W., Wowk, M., Sheeran, F., Bailey, M., ... & Pepe, S. (2005). Coenzyme Q10 therapy before cardiac surgery improves mitochondrial function and in vitro contractility of myocardial tissue. The Journal of Thoracic and Cardiovascular Surgery, 129(1), 25–32.

17. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., ... & Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. Journal of the National Cancer Institute, 82(13), 1107–1112.

18. Smith, M. C., Crist, R. M., Clogston, J. D., & McNeil, S. E. (2017). Zeta potential: A case study of cationic, anionic, and neutral liposomes. Analytical and Bioanalytical Chemistry, 409(24), 5779–5787.

19. Swift, L. M., & Sarvazyan, N. (2000). Localization of dichlorofluorescin in cardiac myocytes: Implications for assessment of oxidative stress. American Journal of Physiology-Heart and Circulatory Physiology, 278(3), H982–H990.

20. World Health Organization. (2021). Cardiovascular diseases (CVDs). Retrieved from who.int fact sheets.

21. Wu, D., & Yotnda, P. (2011). Induction and testing of hypoxia in cell culture. Journal of Visualized Experiments, (54), 2899.

22. Zozina, V. I., Covantev, S., Goroshko, O. A., Krasnykh, L. M., & Kukes, V. G. (2018). Coenzyme Q10 in cardiovascular and metabolic diseases: Current state of the problem. Current Cardiology Reviews, 14(3), 164–174.