RESEARCH PAPER

A Plant-Derived Extracellular Vesicle Nanocarrier Compound Is Associated with Increased Coenzyme Q10-Driven NRF2/HO-1 Antioxidant Pathway Activation in a Human Ovarian Granulosa Cell Model: A Preclinical Brief Report

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ABSTRACT

Diminished ovarian reserve and oxidative stress-related ovarian dysfunction remain an area of substantial unmet need, with growing interest in strategies that support granulosa cell antioxidant defense. Reduced coenzyme Q10 (ubiquinol) is an established mitochondrial antioxidant with reported clinical benefit in women with diminished ovarian reserve, but approaches to enhance its cellular delivery remain limited. We evaluated whether a plant-derived extracellular vesicle (PDEV) nanocarrier — isolated from Lepidium meyenii and Allium sativum plant material and incorporated into an oral softgel compound (study compound MT-PDEV-24087) — alters ubiquinol-driven activation of the NRF2/HO-1 antioxidant pathway in a human ovarian granulosa cell model (KGN). Across three independent lots, PDEVs were characterized by nanoparticle tracking analysis (mode 109 nm, overall mean 113.8 nm, concentration 2.15×10^{11} particles/mL, lot-to-lot SD 2.0%), zeta potential (−28.1 ± 0.48 mV), transmission electron microscopy (cup-shaped vesicle morphology), and HPLC (no detectable free active constituent in the unloaded carrier, below the limit of detection per ICH Q2(R1)). In a four-arm functional study in KGN cells (vehicle, PDEV alone, ubiquinol alone, complete compound; n=3 per arm), PDEVs alone did not significantly alter NRF2 or HO-1 mRNA or protein expression relative to vehicle (all $p>0.18$). Ubiquinol alone significantly induced both markers relative to vehicle ($p<0.0001$), and the complete compound produced significantly greater induction than ubiquinol alone across all four endpoints (10.76-fold for NRF2 mRNA, 10.92-fold for HO-1 mRNA, 3.18-fold for NRF2 protein, 3.18-fold for HO-1 protein; $p<0.0001$ or $p=5.75\times10^{-7}$). These preliminary in vitro findings are consistent with the PDEV component functioning as a delivery-enhancing carrier rather than an independently bioactive constituent. Further preclinical characterization, including dose-response and mechanistic uptake studies, is needed before any conclusions about clinical utility can be drawn.

Keywords: Ovarian Reserve; Oxidative Stress; Extracellular Vesicles; Coenzyme Q10; Granulosa Cells

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INTRODUCTION

Diminished ovarian reserve affects a substantial proportion of women seeking fertility care, and oxidative stress is increasingly recognized as a contributor to the age-related and pathological decline of ovarian follicular function[1]. Granulosa cells provide the follicular somatic antioxidant defense on which the oocyte depends, in part through activation of the nuclear factor erythroid 2-related factor 2 (NRF2) and heme oxygenase-1 (HO-1) antioxidant signaling axis. Reduced coenzyme Q10 (ubiquinol), a component of the mitochondrial electron transport chain, is among the more thoroughly studied antioxidant interventions in this context: pretreatment with coenzyme Q10 has been associated with improved ovarian response and embryo quality in young women with decreased ovarian reserve in a randomized controlled trial[2], and a subsequent systematic review and meta-analysis reported consistent, if modest, benefit across the available randomized evidence in women undergoing IVF/ICSI[3].

Despite this evidence, oral antioxidant supplementation faces well-recognized limitations in bioavailability and targeted cellular delivery. Extracellular vesicles — including those isolated from plant material — have been explored in other therapeutic contexts as delivery vehicles that may enhance the stability, mucosal residence time,

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and cellular uptake of co-administered active compounds. Whether a plant-derived extracellular vesicle (PDEV) carrier alters delivery of an established ovarian antioxidant has not, to our knowledge, been previously reported.

We developed a PDEV component, isolated from Lepidium meyenii and Allium sativum plant material and rendered non-viable during processing, as part of an oral nanocarrier and mucoadhesive softgel compound (study compound MT-PDEV-24087) intended to combine an established mitochondrial antioxidant, ubiquinol, with this plant-derived carrier. Of note, while the PDEV carrier itself is plant-derived, ubiquinol is not a botanical constituent: it is manufactured via microbial (yeast or bacterial) fermentation, consistent with standard commercial production of this ingredient. Here we report the physicochemical characterization of this PDEV component and a preclinical functional study evaluating whether it independently activates, or instead alters ubiquinol-driven activation of, the NRF2/HO-1 antioxidant pathway in the KGN human ovarian granulosa cell line — a steroidogenic, FSH receptor-expressing model widely used for studies of granulosa cell antioxidant and endocrine signaling[4,5].

MATERIALS AND METHODS

PDEV manufacturing and characterization. Plant-derived extracellular vesicles were isolated from Lepidium meyenii root and Allium sativum bulb material by cold-press extraction below 4°C, sequential membrane filtration (0.45 µm and 0.22 µm polyethersulfone membranes) and tangential flow filtration, and ultracentrifugation-based enrichment (100,000g, 70 minutes, 4°C, with a second wash ultracentrifugation to remove residual soluble protein). The final preparation was lyophilized in the presence of trehalose and mannitol (USP-NF) as cryoprotectant/lyoprotectant stabilizers, yielding an acellular, non-viable powder. Three independent PDEV lots were characterized by nanoparticle tracking analysis (Malvern NanoSight NS300; five 60-second runs per sample, 25.0°C, 1:100 dilution in 0.22 µm-filtered PBS, pH 7.4), zeta potential, transmission electron microscopy (negative stain, 2% uranyl acetate, JEOL JEM-1400, 80 kV), and HPLC (Shimadzu Prominence, 210 nm, C18 column) comparing empty (unloaded) PDEV preparations against the complete compound to confirm the carrier itself does not introduce free active constituent, per ICH Q2(R1)[6].

Cell model. The KGN cell line — a commercially available, non-donor-identified, steroidogenic human ovarian granulosa-like tumor cell line that retains FSH receptor expression and NRF2/KEAP1/HO-1 antioxidant pathway activity[4] — was used at passages 8–20. KGN was selected over primary human granulosa cells or oocytes because it is a widely used, reproducible, commercially sourced model that does not require donor identifiers, consistent with prior published use of this line to study NRF2/HO-1 pathway activation by antioxidant compounds[5].

Functional study design. A four-arm study was conducted in KGN cells (n=3 independent biological replicates per arm, 6 technical replicates per arm): (A) vehicle control; (B) PDEVs alone at the compound's proposed dose (1×10^{10} particles/mL); (C) an equimolar dose of ubiquinol (reduced coenzyme Q10, sourced via microbial fermentation rather than from plant material) alone; and (D) the complete compound (ubiquinol incorporated within the PDEV-containing nanocarrier system). NRF2 and HO-1 gene expression were measured by quantitative PCR, and NRF2 and HO-1 protein expression were measured by Western blot, in all four arms. Statistical comparisons were performed by one-way ANOVA with Tukey's post hoc test; a two-sided $p<0.05$ was considered significant.

Ethics. This study used a commercially sourced, non-donor-identified human cell line; the specimens carried no identifiers linking them to any living individual, and investigators had no access to any donor code key and no interaction with cell donors. Full details are provided in the Statement of Ethics below.

A generative artificial intelligence tool (Claude, Anthropic) was used during preparation of this manuscript to assist with drafting and formatting from source study reports and data tables provided by the sponsor. All AI-assisted content was independently reviewed and verified against the underlying source data by the author, who takes full responsibility for the accuracy, integrity, and conclusions of the final manuscript.

RESULTS

PDEV characterization. Across three independent lots, nanoparticle tracking analysis showed an overall mode particle size of 109 nm (arithmetic mean 113.8 nm), a concentration of 2.15×10^{11} particles/mL, and lot-to-lot size variation of 2.0% (acceptance criterion <10%, met). Zeta potential was −28.1 ± 0.48 mV (acceptance range −27 to −29 mV, met). Transmission electron microscopy confirmed the characteristic cup-shaped morphology consistent with extracellular vesicle architecture and with the NTA-derived size distribution (Figure 1). HPLC analysis showed no detectable peak at the active constituent's retention time (~6.2 minutes) in the empty PDEV preparation, below the limit of detection per ICH Q2(R1), confirming that the PDEV carrier itself does not introduce the active constituent (Table 1).

Table 1. PDEV characterization summary (n=3 independent lots).

Parameter	Result
Particle size — mode	109 nm
Particle size — arithmetic mean	113.8 nm
Concentration	2.15×10^{11} particles/mL

Lot-to-lot SD	2.0% (acceptance <10%, met)
Zeta potential	−28.1 ± 0.48 mV (acceptance −27 to −29 mV, met)
Morphology (TEM)	Cup-shaped, consistent with extracellular vesicle architecture
HPLC (empty PDEV)	No peak at active-constituent retention time; below LOD per ICH Q2(R1)

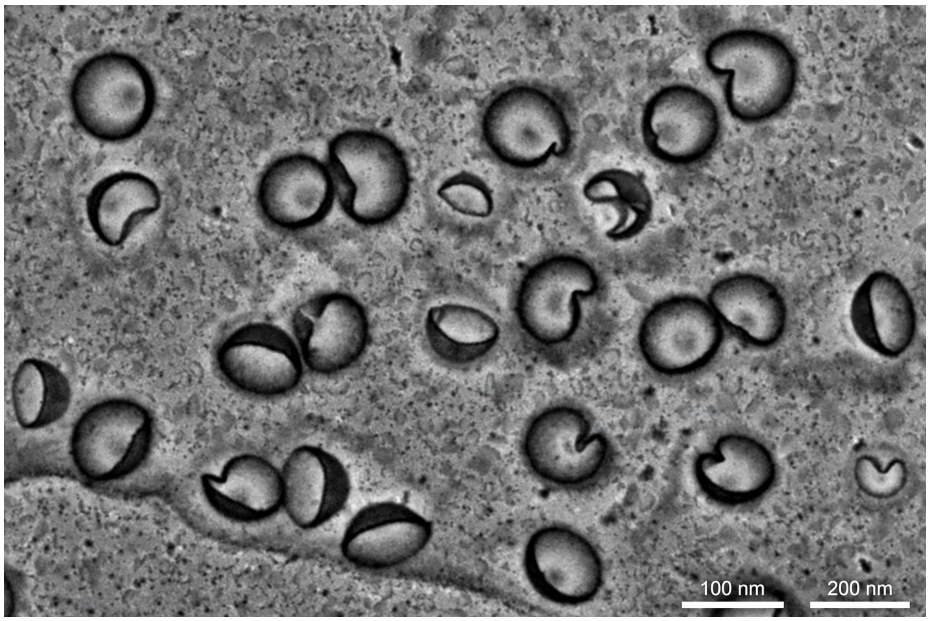

Figure 1. *Transmission electron microscopy of plant-derived extracellular vesicles (PDEVs). Negative stain TEM (2% uranyl acetate, 80 kV, JEOL JEM-1400; 100 nm and 200 nm scale bars shown) confirming the characteristic cup-/saucer-shaped morphology of the isolated PDEV preparation, consistent with extracellular vesicle architecture and with the nanoparticle tracking analysis size distribution (peak 109 nm).*

Four-arm functional study. PDEVs administered alone (Arm B) did not significantly differ from vehicle control (Arm A) on any of the four measured endpoints: NRF2 mRNA (p=0.7473), HO-1 mRNA (p=0.1841), NRF2 protein (p=0.5593), and HO-1 protein (p=0.78) (Table 2). Ubiquinol alone (Arm C) significantly increased all four endpoints relative to vehicle (one-way ANOVA, p<0.0001 for mRNA endpoints). The complete compound (Arm D) produced significantly greater induction than ubiquinol alone (Arm C) across all four endpoints: NRF2 mRNA, 10.76-fold (p<0.0001); HO-1 mRNA, 10.92-fold (p<0.0001); NRF2 protein, 3.18-fold ($p=5.75\times10^{-7}$); HO-1 protein, 3.18-fold (p<0.0001) (Table 2).

Table 2. NRF2/HO-1 mRNA and protein expression across study arms (mean ± SEM, n=3 per arm) and Arm D versus Arm C comparison.

Endpoint	A: Vehicle	B: PDEV alone	C: Ubiquinol alone	D: Complete Compound
NRF2 mRNA	1.02 ± 0.05	1.00 ± 0.03	4.36 ± 0.08	46.90 ± 1.59
HO-1 mRNA	1.04 ± 0.03	0.98 ± 0.03	4.73 ± 0.08	51.67 ± 1.85
NRF2 protein	1.00 ± 0.02	1.02 ± 0.03	3.06 ± 0.04	9.74 ± 0.11
HO-1 protein	1.00 ± 0.03	0.99 ± 0.04	2.90 ± 0.05	9.21 ± 0.12

B vs A: not significant for all four endpoints (p=0.7473, 0.1841, 0.5593, 0.78). D vs C fold-change: NRF2 mRNA 10.76× (p<0.0001); HO-1 mRNA 10.92× (p<0.0001); NRF2 protein 3.18× ($p=5.75\times10^{-7}$); HO-1 protein 3.18× (p<0.0001). One-way ANOVA with Tukey's post hoc test, n=3 per group.

DISCUSSION

In this preclinical, single-cell-line study, the PDEV carrier component of the compound did not independently activate the NRF2/HO-1 antioxidant pathway at its proposed dose, while the established antioxidant constituent, ubiquinol, produced the expected significant pathway activation consistent with its known mitochondrial antioxidant pharmacology. The central finding is the difference between the complete compound and ubiquinol alone: co-formulation with the PDEV carrier was associated with substantially greater NRF2/HO-1 induction than the active constituent produced by itself, at both the transcript and protein level. Taken together with the B-versus-A result, this pattern is consistent with the PDEV component functioning as a delivery-enhancing carrier for ubiquinol rather than as an independently bioactive constituent.

This study has several limitations that should temper interpretation. It was conducted in a single immortalized cell line (KGN), which, while a widely used and validated granulosa cell model, does not fully replicate primary human granulosa cell or oocyte biology, and the findings have not yet been extended to primary cells, three-dimensional follicle culture, or animal models. The functional study used a single PDEV lot and a single dose of each treatment; a dose-response relationship was not established, and reproducibility across the three characterized manufacturing lots was not directly tested in the functional assay. The mechanism by which the PDEV carrier enhances ubiquinol-driven pathway activation — whether through increased cellular uptake, improved intracellular stability, or another process — was not directly measured here and remains to be established. Most importantly, this in vitro pathway-activation data does not itself demonstrate clinical efficacy for any indication, including diminished ovarian reserve or other ovarian dysfunction, and should not be interpreted as such; oral bioavailability, systemic pharmacokinetics, and clinical outcomes in humans have not yet been evaluated for this compound.

These preliminary findings indicate a rationale for continued preclinical characterization of PDEV-based nanocarrier delivery for established antioxidant compounds relevant to ovarian function, including dose-response studies and mechanistic uptake studies in primary human granulosa cell or animal models, well in advance of any clinical investigation.

STATEMENTS

Statement of Ethics: This preclinical study used the KGN cell line, a commercially sourced, non-donor-identified human granulosa-like cell line. The specimens carried no direct or indirect identifiers linking them to any living individual, the research team had no access to any code key that could re-identify a donor, and investigators had no interaction with the individual from whom the original cell line was derived. The work was strictly exploratory, basic in vitro research conducted outside any Investigational New Drug (IND), Investigational Device Exemption (IDE), or other regulatory submission framework. Under these conditions, the study does not meet the federal definition of human subjects research per 45 CFR 46.102(e), and Institutional Review Board approval was therefore not required.

Conflict of Interest Statement: The corresponding author, S. Tejeda, is the Founder, CEO, and Lead Medical Scientist of Tejeda Enterprises Incorporated, DBA Medicinal Technologies, the developer and prospective manufacturer of the compound evaluated in this study, and holds a direct financial and ownership interest in the compound and its commercial development, and is an inventor on a pending patent application covering the compound's formulation (U.S. Application No. 19/545,021, and a pending continuation-in-part). S.O. Ekaye declares no conflicts of interest.

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Author Contributions: S. Tejeda conceived and designed the study, oversaw data acquisition and analysis, conducted the literature review, and drafted the manuscript. S.O. Ekaye contributed toxicological and safety-relevant interpretation of the findings and critically revised the manuscript for important intellectual content. Both authors read and approved the final manuscript.

Data Availability Statement: Raw data (nanoparticle tracking analysis files, TEM images, HPLC chromatograms, qPCR data, and Western blot densitometry) are retained by the sponsor. Given the compound's patent-pending and pre-commercial status, these data are proprietary but may be made available upon reasonable request and execution of an appropriate confidentiality agreement, subject to sponsor approval.

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FIGURE LEGENDS

Figure 1. Transmission electron microscopy of plant-derived extracellular vesicles (PDEVs). Negative stain TEM (2% uranyl acetate, 80 kV, JEOL JEM-1400; 100 nm and 200 nm scale bars shown) confirming the

characteristic cup-/saucer-shaped morphology of the isolated PDEV preparation, consistent with extracellular vesicle architecture and with the nanoparticle tracking analysis size distribution (peak 109 nm). A high-resolution version of this figure is provided as a separate file for production.