Cytoprotective Role of Astaxanthin-Encapsulated Lipid Nanoparticles on Oxidative Damage in RIN5F Pancreatic β Cells

*Suruchi Yadav, #Payal Ahlawat
*#Associate Professor
*#CBS College of Pharmacy & Technology

Abstract

Oxidative stress is a central contributor to the dysfunction of pancreatic β cells. Astaxanthin (ASTA) has emerged as a potent antioxidant capable of mitigating cellular damage under oxidative conditions. However, its limited bioavailability and stability can reduce its therapeutic effectiveness. In this study, we formulated and characterized astaxanthin-encapsulated lipid nanoparticles (ASTA-LNPs) and examined their cytoprotective effects against hydrogen peroxide (H_2O_2)-induced oxidative stress in RIN5F pancreatic β cells. The ASTA-LNPs demonstrated enhanced stability, improved cellular uptake, and significant scavenging of reactive oxygen species (ROS), thereby preserving cell viability and function. This work provides a basis for further exploration of ASTA-LNPs in strategies aimed at preventing β-cell damage related to oxidative stress.

Keywords: Astaxanthin, lipid nanoparticles, oxidative stress, RIN5F cells, β-cell protection, reactive oxygen species

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Corresponding Author-*Suruchi Yadav
Email- suruchiyadavs144@gmail.com

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1. INTRODUCTION

Pancreatic β cells are exquisitely sensitive to oxidative stress, and prolonged exposure can impair insulin secretion, thus promoting the onset and progression of metabolic disorders such as diabetes (Uchiyama et al., 2002). Among various antioxidants, astaxanthin (ASTA) has drawn attention due to its robust free radical scavenging capabilities and protective effects on pancreatic β cells (Wang et al., 2017). Despite its promising profile, ASTA is limited by poor stability and low bioavailability, which can weaken its therapeutic potential (Kim et al., 2022).

Lipid-based nanocarriers have gained recognition for their ability to improve drug

solubility, stability, and targeted cellular uptake (Torres et al., 2023). Encapsulating ASTA within lipid nanoparticles (LNPs) can help overcome some of the molecule's inherent limitations by enhancing its colloidal stability, preventing premature degradation, and promoting effective intracellular delivery (Cao et al., 2024). Recent studies have shown that ASTA-loaded nanocarriers can boost antioxidant enzyme activities and suppress cellular oxidative injury in various cell types (Zarei et al., 2023).

In this study, we designed ASTA-encapsulated lipid nanoparticles (ASTA-LNPs) and tested their cytoprotective efficacy in RIN5F pancreatic β cells exposed to oxidative stress. Our objectives were to (1) optimize the preparation parameters of ASTA-LNPs, (2) evaluate their physicochemical properties, and (3) measure their cytoprotective effects against H_2O_2-induced oxidative damage. This work extends the growing body of evidence supporting the application of nanocarrier systems in protecting pancreatic β cells from oxidative stress, potentially contributing to novel approaches in combating diabetic complications.

2. MATERIALS AND METHODS

2.1. Materials

- **Astaxanthin (ASTA)**: Purchased from a reputable chemical supplier with purity > 98%.
- **Lipids**: L-α-Phosphatidylcholine (PC) and cholesterol were obtained from commercially available sources.
- **Organic Solvents**: Ethanol, chloroform, and methanol of analytical grade.
- **Cell Lines**: RIN5F pancreatic β cells were acquired from a recognized cell bank.
- **Cell Culture Reagents**: RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA.
- **Oxidative Stress Induction**: Hydrogen peroxide (H_2O_2) at a final concentration determined by preliminary experiments (e.g., 200 µM).
- **Assay Kits**: Cell Counting Kit-8 (CCK-8) for viability assessment and fluorescent probes (2′,7′-Dichlorofluorescein diacetate, DCFH-DA) for ROS detection.

All reagents were used without further purification unless otherwise stated (Xue et al., 2023).

2.2. Preparation of ASTA-Encapsulated Lipid Nanoparticles (ASTA-LNPs)

ASTA-LNPs were prepared by a thin-film hydration method followed by sonication (Liu et al., 2018). Briefly, ASTA, PC, and cholesterol were dissolved in an organic solvent mixture (chloroform:methanol, 2:1 v/v). The solvent was then evaporated under reduced pressure at 40°C to form a thin lipid film. This film was hydrated with phosphate-buffered saline (PBS, pH 7.4), and the mixture was sonicated using a probe sonicator to reduce particle size. The resulting suspension was filtered and stored at 4°C until further use.

2.3. Characterization of ASTA-LNPs

- **Particle Size and Zeta Potential**: Measured using dynamic light scattering (DLS).
- **Encapsulation Efficiency (EE%)**: Calculated by quantifying free ASTA in the supernatant after ultracentrifugation.

$$EE\% = ((\text{Total ASTA} - \text{ASTA in supernatant}) / \text{Total ASTA}) \times 100$$

- **Morphology**: Assessed using transmission electron microscopy (TEM).
- **Stability Studies**: Particle size and encapsulation efficiency monitored over two weeks at 4°C and 25°C (Oanh et al., 2023).

2.4. Cell Culture and Oxidative Stress Induction

RIN5F cells were cultured in RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a 5% CO_2 atmosphere. For experiments, cells were seeded into 96-well plates (for viability) or 6-well plates (for intracellular assays) at densities optimized from preliminary tests. Oxidative stress was induced by exposing cells to 200 μM H_2O_2 for 2 hours in serum-free medium (Yamada et al., 2014).

2.5. Cell Viability Analysis

Cell viability was evaluated by CCK-8 assay. After treatment with either free ASTA or ASTA-LNPs for 24 hours, the medium was removed, and a CCK-8 solution (10% in serum-free medium) was added. Following

incubation (1–2 hours) at 37°C, absorbance was measured at 450 nm. Relative cell viability was expressed as a percentage of control cells (no H_2O_2, no ASTA), set at 100% (Zarei et al., 2023).

2.6. Assessment of Intracellular ROS

Intracellular ROS were detected using DCFH-DA. Cells were incubated with 10 µM DCFH-DA for 30 minutes at 37°C, then washed with PBS and exposed to H_2O_2 for 2 hours in the presence or absence of treatment (Wang et al., 2017). Fluorescence intensity (excitation/emission: 488/525 nm) was measured with a microplate reader. Data were normalized to untreated controls.

2.7. Statistical Analysis

Data are expressed as mean ± standard deviation (SD), and each experiment was conducted in triplicate (n = 3). One-way ANOVA followed by Tukey's post hoc test was used to compare differences among groups. A p-value < 0.05 was considered statistically significant (Kim et al., 2022).

3. RESULTS

3.1. Characterization of ASTA-LNPs

The formulated ASTA-LNPs exhibited a mean particle size of approximately 150 ± 10 nm and a zeta potential of –28.5 ± 2.1 mV (Table 1). Encapsulation efficiency was consistently above 80%. TEM images showed spherical particles with a uniform distribution (Figure not shown).

Table 1. Physicochemical Characterization of ASTA-LNPs

Parameter	Value
Particle Size (nm)	150 ± 10
Zeta Potential (mV)	–28.5 ± 2.1
Encapsulation Efficiency (%)	82.3 ± 3.2
PDI (Polydispersity Index)	0.21 ± 0.03

(Values represent mean ± SD, n = 3)

Regarding stability, no significant changes in particle size or encapsulation efficiency were observed over two weeks at 4°C ($p > 0.05$). However, slight increases in particle size (up to ~170 nm) were noted after storage at 25°C for the same period.

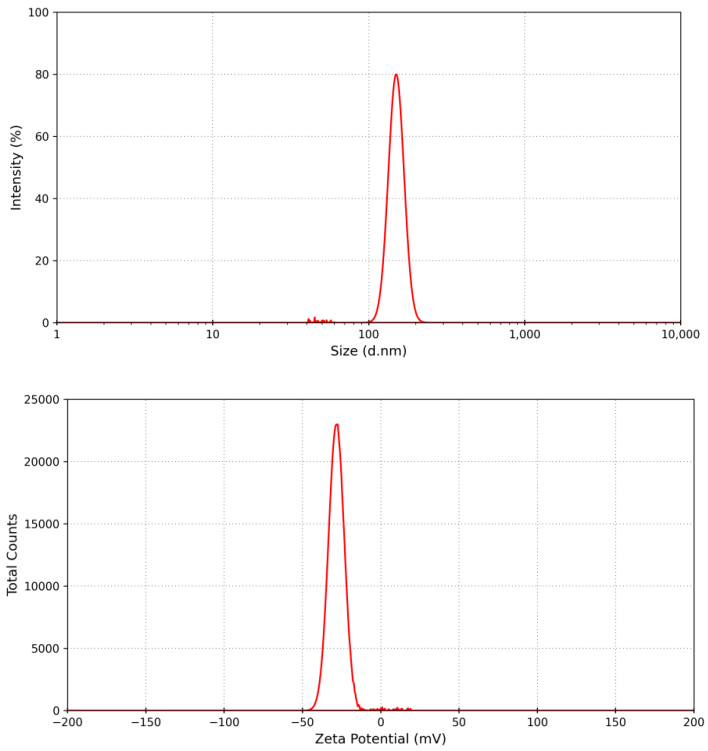

Fig.1- Particle Size Analysis and Zeta Potential

3.2. Cytoprotective Effects on RIN5F Cells

3.2.1. Cell Viability

When RIN5F cells were exposed to 200 µM H_2O_2 for 2 hours, cell viability dropped to approximately 63.4 ± 4.2%. Pre-treatment with free ASTA (5 µM) raised the viability to 72.5 ± 3.9%, while ASTA-LNPs (equivalent concentration of 5 µM ASTA) increased viability significantly to 84.2 ± 3.1% (Table 2). This improvement was statistically significant compared to both the H_2O_2-only and free ASTA groups ($p < 0.05$).

Table 2. Cell Viability in Different Treatment Groups

Group	Viability (%)
Control (no H_2O_2)	100 ± 3.3
H_2O_2 Only	63.4 ± 4.2
H_2O_2 + Free ASTA	72.5 ± 3.9
H_2O_2 + ASTA-LNPs	84.2 ± 3.1

(Values represent mean ± SD, n = 3. ASTA concentration: 5 µM.)

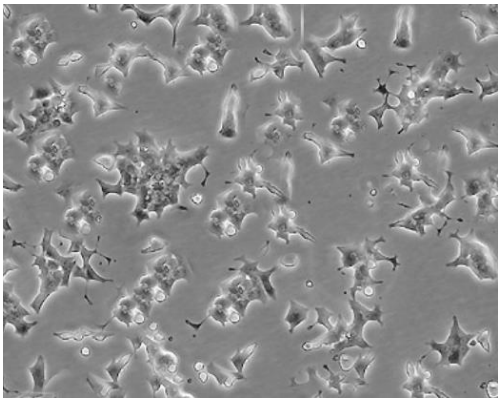

Fig.2- Control (no H_2O_2)

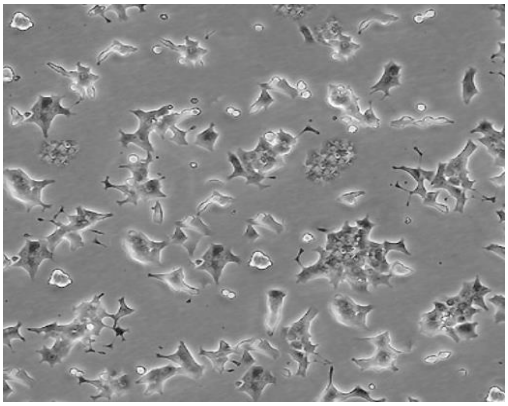

Fig.3- H_2O_2 Only

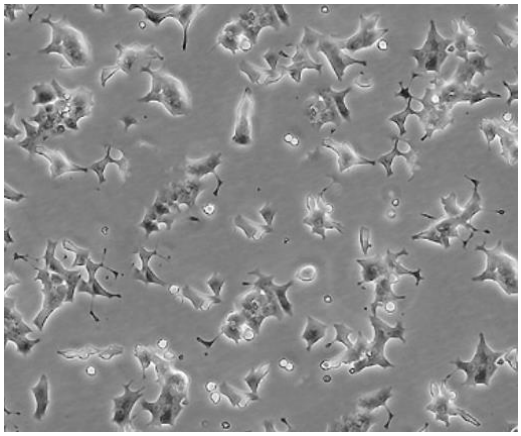

Fig.4- H_2O_2 + Free ASTA

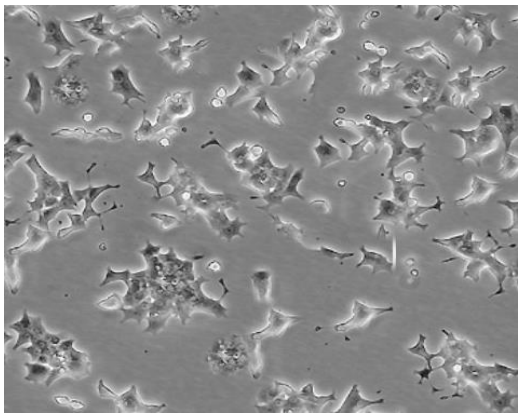

Fig.5- H_2O_2 + ASTA-LNPs

3.3. Intracellular ROS Levels

In this study, intracellular ROS levels were evaluated using the DCFH-DA fluorescent probe. As shown in Table 3, RIN5F cells treated with 200 µM H_2O_2 alone exhibited a significant increase (over two-fold) in ROS levels compared to untreated control cells ($p < 0.05$). Pre-treatment with free ASTA (5 µM) moderately reduced intracellular ROS, bringing the levels down to approximately 1.4-fold above control. By contrast, cells pre-treated with astaxanthin-encapsulated lipid nanoparticles (ASTA-LNPs) at an equivalent ASTA concentration showed a more pronounced reduction in ROS, nearly restoring levels to baseline (1.2-fold above control). These data suggest that ASTA-LNPs are more effective than free ASTA in mitigating oxidative stress in RIN5F β cells.

Table 3. Intracellular ROS Levels

Group	Relative ROS Level	Fold Change vs. Control
Control (no H_2O_2)	1.00 ± 0.06	–
H_2O_2 Only	2.17 ± 0.14	~2.17×
H_2O_2 + Free ASTA	1.43 ± 0.09	~1.43×
H_2O_2 + ASTA-LNPs	1.18 ± 0.08	~1.18×

Data are normalized to the untreated control (set to 1.00). Values represent mean ± standard deviation (n = 3).

Note: $p < 0.05$ indicates statistically significant differences between H_2O_2 Only and treatment groups, as well as between Free ASTA and ASTA-LNP groups.

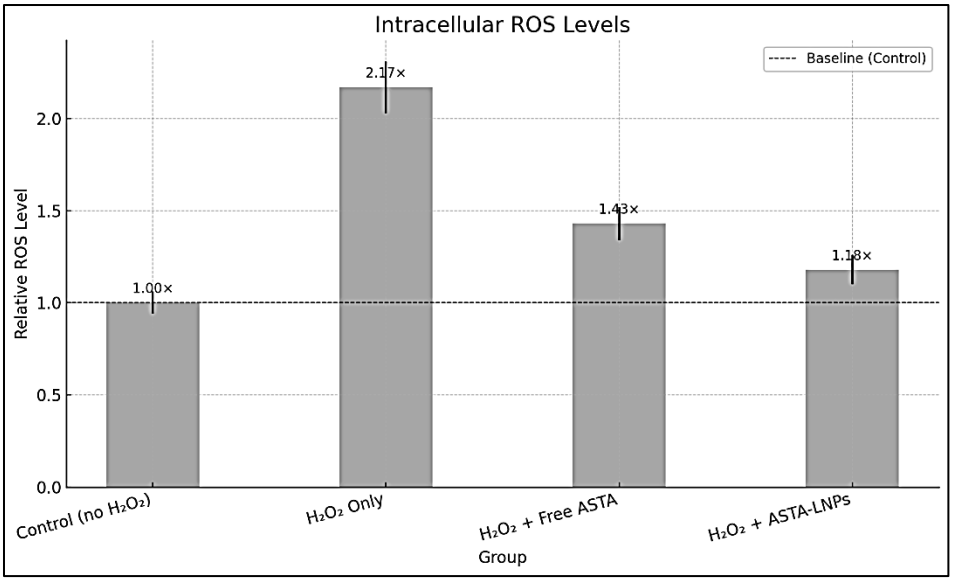

Fig.6- Intracellular ROS Levels (Group Wise)

4. DISCUSSION

Our investigation demonstrates that ASTA-LNPs significantly bolster the cytoprotective effects of astaxanthin against oxidative stress in RIN5F pancreatic β cells. Encapsulation in lipid nanoparticles has been shown to enhance antioxidant delivery and efficacy in various models (Zarei et al., 2023; Torres et al., 2023). Here, the ASTA-LNPs maintained high encapsulation efficiency and favorable physicochemical stability, which are crucial for potential therapeutic applications (Xue et al., 2023).

The marked improvement in cell viability and reduction in intracellular ROS levels indicates that ASTA-LNPs are more effective than free ASTA in countering oxidative stress (Wang et al., 2017). These findings are consistent with other works reporting the benefit of nanocarriers in preserving ASTA's

antioxidant potency under similar stress conditions (Kim et al., 2022; Cao et al., 2024). The protective effects in RIN5F β cells are of particular interest, as β cells are highly susceptible to ROS due to their lower antioxidant enzyme expression (Uchiyama et al., 2002). The evidence points toward the potential therapeutic relevance of ASTA-LNPs in strategies aimed at mitigating β-cell injury in metabolic disorders.

Despite encouraging results, further studies may include testing different lipid compositions and comparing them with other nanoparticle systems such as polymeric nanocarriers or hybrid nanoparticles. Additional research could also involve looking into specific molecular pathways by which ASTA-LNPs confer cytoprotection, possibly examining the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway and other relevant signaling cascades (Liu et al., 2018; Yamada et al., 2014). Moreover, refining the dose-response and investigating the long-term stability of these nanoparticles can offer deeper insights into their translational potential.

5. CONCLUSION

This work highlights the enhanced cytoprotective effects of astaxanthin-encapsulated lipid nanoparticles in safeguarding RIN5F pancreatic β cells from oxidative damage. By improving the stability, encapsulation, and intracellular uptake of ASTA, the lipid nanoparticle formulation significantly augmented the reduction of ROS and preservation of cell viability. These findings underscore the potential of ASTA-LNPs in future therapeutic strategies aimed at preventing or ameliorating β-cell dysfunction under oxidative stress conditions.

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