EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
IF = 9.2 www.in-academy.uz/index.php/ejmns

IN VITRO EVALUATION OF CYTOTOXICITY AND BIOCOMPATIBILITY OF GRAPE SEED EXTRACT-LOADED SNEDDS GEL IN HACAT AND CACO-2 CELL MODELS

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https://doi.org/10.5281/zenodo.19565019

ARTICLE INFO

Received: 06th April 2026
Accepted: 13th April 2026
Online: 14th April 2026

KEYWORDS

SNEDDS, topical delivery, cytotoxicity, HaCaT, Caco-2, MTT assay, biocompatibility, antioxidant activity.

ABSTRACT

Self-nanoemulsifying drug delivery systems (SNEDDS) have emerged as promising platforms for improving the solubility and bioavailability of poorly water-soluble compounds, particularly plant-derived bioactives. In the present study, SNEDDS-based formulations were developed and incorporated into a Carbopol hydrogel and further combined with a non-ionic hydrophilic ointment base to obtain a semi-solid delivery system suitable for topical application.

The cytotoxicity and biocompatibility of the developed systems were investigated using the MTT assay on HaCaT human keratinocytes and Caco-2 intestinal epithelial cell lines.

The results demonstrated that the SNEDDS-based formulations provided effective dispersion of plant-derived active compounds and exhibited notable antioxidant activity. Texture analysis confirmed suitable mechanical properties for topical administration. Cytotoxicity studies revealed high cell viability (>90%) in HaCaT cells at lower concentrations, indicating good dermal compatibility. In contrast, a concentration-dependent decrease in cell viability was observed in Caco-2 cells, with significant cytotoxic effects at higher concentrations, likely due to surfactant-mediated membrane interactions and enhanced cellular uptake.

Overall, the developed SNEDDS-loaded gel and ointment systems exhibit promising physicochemical and biological properties, supporting their potential application as advanced topical delivery platforms for bioactive compounds. However, careful optimization of concentration is required to ensure safety and minimize cytotoxic effects.

Introduction. The development of effective delivery systems for poorly water-soluble bioactive compounds remains a significant challenge in pharmaceutical and biomedical research. Many plant-derived compounds, including polyphenols such as those found in grape seed extract, exhibit potent antioxidant and therapeutic properties; however, their clinical application is often limited by low aqueous solubility, poor stability, and insufficient bioavailability [1,2].

Self-nanoemulsifying drug delivery systems (SNEDDS) have emerged as promising platforms to overcome these limitations. SNEDDS are isotropic mixtures of oils, surfactants, and co-solvents that spontaneously form fine oil-in-water nanoemulsions upon contact with aqueous media [3,4]. This unique property enhances drug solubilization, improves dispersion, and facilitates cellular uptake, making SNEDDS particularly suitable for the delivery of lipophilic and poorly soluble compounds [5].

For topical applications, the incorporation of SNEDDS into semi-solid dosage forms such as hydrogels provides additional advantages, including improved application properties, prolonged residence time, and enhanced patient compliance [6]. Carbopol-based hydrogels are widely used due to their favorable rheological characteristics, biocompatibility, and ability to form stable formulations [7]. When combined with SNEDDS, such systems may further enhance the penetration and effectiveness of bioactive compounds [8].

Despite these benefits, the presence of surfactants and co-solvents in SNEDDS formulations raises concerns regarding potential cytotoxicity, particularly at higher concentrations [9]. Therefore, the evaluation of biocompatibility is essential during formulation development. In vitro cell-based assays provide a reliable and reproducible approach for assessing cytotoxic effects [10]. HaCaT human keratinocytes are commonly used as a model for skin compatibility, while Caco-2 intestinal epithelial cells serve as a model for evaluating epithelial barrier interactions and potential systemic exposure [11,12].

Among available methods, the MTT assay is widely used to assess cell viability based on mitochondrial metabolic activity, allowing quantitative evaluation of dose-dependent cytotoxic effects [13].

The aim of this study was to develop SNEDDS-based gel formulations containing grape seed extract and to evaluate their cytotoxicity and biocompatibility using MTT assays on HaCaT and Caco-2 cell lines. Particular emphasis was placed on investigating the concentration-dependent effects of the formulations to determine their safety profile and suitability for pharmaceutical applications.

Materials and Method

Materials. Polysorbate 80 (Tween 80), Transcutol® P, oleic acid, isopropyl myristate, ethanol, and purified water (Aqua purificata) were used for the preparation of self-nanoemulsifying drug delivery systems (SNEDDS). Carbopol® 934P was used as a gelling agent, and triethanolamine (TEA) served as a neutralizing agent.

Grape seed extract was used as the model bioactive compound. All reagents were of analytical grade and used without further purification.

Dulbecco's Modified Eagle Medium (DMEM), phosphate-buffered saline (PBS), MTT reagent (tetrazolium bromide), and Triton X-100 were used for cell culture and cytotoxicity studies.

Preparation of SNEDDS-gel. The SNEDDS formulation was prepared by mixing oleic acid and isopropyl myristate as the oil phase with Tween 80, Transcutol® P, and ethanol as surfactant and co-surfactant components under magnetic stirring until a clear system was obtained.

Grape seed extract was incorporated into the formulation and homogenized to ensure complete dispersion. The SNEDDS system was then incorporated into a Carbopol® 934P hydrogel base under continuous stirring. Gel formation was induced by dropwise addition of triethanolamine until a homogeneous semi-solid formulation was obtained.

Preparation of samples for cytotoxicity testing. The prepared SNEDDS-gel formulations were diluted in phosphate-buffered saline (PBS) to obtain a series of concentrations (0.1%, 0.01%, 0.001%, and 0.0001%) for biological evaluation.

PBS was used as a negative control, while 1% Triton X-100 served as a positive control to induce complete cell death.

Cell culture. HaCaT human keratinocytes and Caco-2 human intestinal epithelial cells were used as in vitro models. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) under standard conditions (37 °C, 5% CO_2, humidified atmosphere).

Cells were seeded into 96-well plates at a density of 1×10^4 cells per well and allowed to attach and grow prior to treatment.

MTT assay. Cell viability was assessed using the MTT assay. After reaching appropriate confluence, cells were exposed to SNEDDS-gel samples at different concentrations. HaCaT cells were incubated with samples for 2 h, while Caco-2 cells were treated for 1 h at 37 °C.

Following treatment, the medium was removed and MTT solution (0.5–5 mg/mL) was added to each well. Cells were incubated for 3 h to allow the formation of formazan crystals. The crystals were dissolved in an isopropanol:HCl solution (25:1), and absorbance was measured at 570 nm (reference: 690 nm) using a microplate reader.

Cell viability (%) was calculated relative to untreated control cells.

Results and Discussion

Cytotoxicity and biocompatibility (MTT assay). The cytotoxicity of the SNEDDS-based gel containing grape seed extract was evaluated using the MTT assay on cultured cells. As shown in Figure 1, the untreated control (PBS) demonstrated 100% cell viability, confirming normal cell metabolic activity, while the positive control (Triton X-100) significantly reduced viability to approximately 10%, indicating complete membrane disruption and validating the assay conditions.

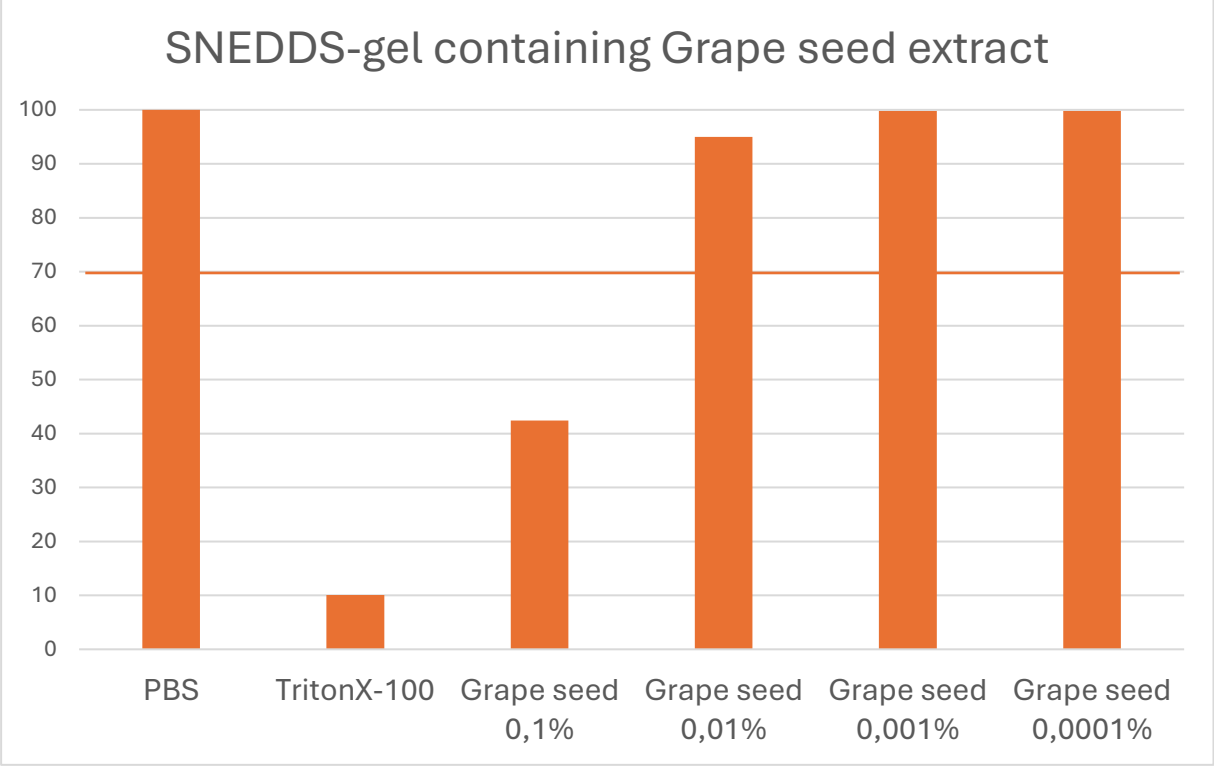

Figure 1. Cell viability (%) of SNEDDS-gel containing grape seed extract at different concentrations evaluated by MTT assay. PBS was used as a negative control and Triton X-100 as a positive control.

The SNEDDS-gel formulation exhibited a clear concentration-dependent effect on cell viability. At the highest concentration (0.1%), cell viability decreased to approximately 42%, indicating moderate cytotoxicity. However, a significant improvement in cell viability was observed with decreasing concentration. At 0.01%, viability increased to approximately 95%, while at 0.001% and 0.0001%, cell viability reached nearly 100%, indicating minimal cytotoxicity.

This trend suggests that the SNEDDS system enhances the interaction of bioactive compounds with cells at higher concentrations, likely due to improved solubilization and cellular uptake facilitated by surfactants. At lower concentrations, however, the formulation demonstrates excellent biocompatibility, preserving cell viability while maintaining functional activity.

Conclusion

In this study, SNEDDS-based gel formulations containing grape seed extract were successfully developed and evaluated for their cytotoxicity and biocompatibility. The results demonstrated that the formulations exhibit a clear concentration-dependent effect on cell viability. At lower concentrations (≤0.001%), the SNEDDS-gel showed excellent biocompatibility, maintaining high cell viability, while higher concentrations (0.1%) induced moderate cytotoxic effects.

The findings indicate that the SNEDDS system effectively enhances the dispersion and biological interaction of plant-derived bioactive compounds, which may contribute to improved functional activity. At the same time, the presence of surfactants highlights the

importance of optimizing formulation concentration to ensure safety.

Overall, the developed SNEDDS-based gel represents a promising delivery platform for topical application of natural antioxidants. The combination of high biocompatibility at optimized concentrations and effective formulation characteristics supports its potential for further pharmaceutical development. Future studies should focus on in vivo evaluation and long-term safety assessment to confirm its clinical applicability.

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