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Synthesis and Cytotoxic Activity of Novel Vit E infused Nanogel Formulation: An In Vitro Study

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KEYWORDS

Vitamin E, cytotoxicity, Chitin, oral cancer cell line, Chitosan, local drug delivery, oral cancer, Nano formulation.

ABSTRACT:

Background: Vitamin E, a group of fat-soluble compounds first discovered in 1922, is valued for its various properties and includes tocopherols and tocotrienols. This study introduces a novel vitamin E-infused nanogel formulated with biodegradable chitosan nanoparticles, which enhances targeted drug delivery. This present study aims to synthesize a novel vitamin E-infused nano gel formulation and to evaluate the cytotoxic effects on the oral cancer cell line.

Materials and Methods: Formulation was prepared by combining 5 grams of Vitamin E powder, 0.5 grams of Chitosan, 0.2 milliliters of Tween 80, and 10 milliliters of Monosodium Phosphate in 90 milliliters of distilled water. This mixture was then heated to 55°C for 30 minutes while stirring with a magnetic stirrer. After heating, the mixture was lyophilized to form a gel. The cytotoxicity of the formulation was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on the oral cancer cell line.

Results: The cytotoxic activity of the vitamin E-infused nanogel was evaluated at various concentrations, including 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 µg/mL, and was compared to both a control and a negative control group. The results showed a dose-dependent decrease in cell viability, indicating significant cytotoxic effects of the formulation on oral cancer cell lines. The formulation has shown high efficacy at a concentration of 120 µg/mL

Conclusion: This study highlights the potential of a vitamin E-infused nanogel with enhanced cytotoxic activity against oral cancer cell lines. Utilizing biodegradable chitosan nanoparticles, the nanogel ensures targeted drug delivery, improves adherence, and reduces side effects. It shows promise as an effective alternative for treating oral potentially malignant disorders and mucocutaneous conditions. Future research should explore its efficacy through in-vivo studies and clinical trials.

1. Introduction

Vitamin E refers to a group of fat-soluble compounds first identified by Evans and Bishop in 1922. These compounds are known for their important antioxidant properties, which support overall health. Vitamin E includes tocochromanols, consisting of tocopherols and tocotrienols, exhibiting the biological activity of d-alpha-tocopherol [1].

Cancer, characterized by uncontrolled cell division, is a leading cause of death worldwide. Alternative treatments, such as cancer immunotherapy, are crucial because traditional chemotherapy and radiotherapy can harm both cancerous and healthy dividing cells. Key immunotherapy methods include monoclonal antibody transfer, adoptive T-cell transfer (ACT), and immune checkpoint therapy. The primary goal of immunotherapy is to enhance the immune system, allowing immune cells to target and destroy cancer cells while maintaining the cancer immunity cycle. vitamin E and its analogs augment anticancer immune response by affecting different key steps of the cancer-immunity cycle [2,3].

Drug delivery systems (DDS) hold significant importance in the medical field, with nanoparticles serving as key components [4]. Chitosan, derived from the natural polysaccharide chitin, is the second most abundant polysaccharide globally after cellulose. Its attributes, including biocompatibility, biodegradability,

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antibacterial activity, and mucoadhesive properties, make chitosan highly versatile for applications in food, cosmetics, textiles, water purification, and medicine field. Chitosan nanoparticles, in particular, are of great interest due to their strong mucoadhesive characteristics and cytotoxic potential [5].

Previous research has demonstrated the cytotoxic properties of vitamin E [6]. This study introduces a novel vitamin E-infused nanogel formulation, offering unique properties that have not been investigated previously, thereby adding an element of innovation. The research aims to further evaluate these effects in an in-vivo context and explore their potential application in treating oral potentially malignant disorders (OPMDs), such as oral lichen planus, as well as other mucocutaneous conditions. Currently, vitamin E is primarily administered orally as capsules or tablets for managing oral lichen planus. However, a topical formulation could improve patient compliance, enable localized drug delivery, and enhance therapeutic outcomes. This study aims on synthesizing a new vitamin E-based nanoformulation and assessing its cytotoxic effects on oral cancer cell lines.

2. Materials and Methods

Materials used

The Nano gel formulation includes 5 g of vitamin E powder (Fig 1), 0.5 g of Chitosan (Fig 2), 0.2 ml of Tween 80 (polysorbate 80) (Fig 3), 10 ml of Monosodium Phosphate (Fig 4), and 90 ml of water.

Preparation of Formulation

A mixture was prepared by dissolving 5 grams of Vitamin E powder, 0.5 grams of Chitosan, 0.2 milliliters of Tween 80, and 10 milliliters of Monosodium Phosphate in 90 milliliters of distilled water. This mixture was heated to 55°C for 30 minutes with continuous magnetic stirring. It was then subjected to 5 minutes of sonication using an ultra-probe sonicator and subsequently lyophilized to create the vitamin E nano gel formulation [figures 5 and 6]. The cytotoxicity of this formulation was evaluated using the oral cancer cell line.

Culture of Oral Cancer Cell Line:

The oral cancer cell line was obtained from the Cell Repository at the National Centre for Cell Science in Pune, India. It was maintained in Dulbecco's Modified Eagle Medium (DMEM) from Himedia, Mumbai, India, supplemented with 10% heat-inactivated fetal bovine serum (FBS). The cultures were incubated in a controlled environment at 37ºC with 5% CO2 and 95% humidity.

Cytotoxicity Assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was conducted to evaluate the anti-cancer effectiveness of the vitamin E-infused nanogel on oral cancer cells. Cells were plated in 96-well microtiter plates and allowed to reach 70% confluency before being treated with varying concentrations of the vitamin E nanogel (ranging from 10 to 100 µg/ml). After a 24-hour incubation at room temperature, the MTT solution was discarded, and 100 µl of DMSO was added to dissolve the purple formazan crystals. Then, 10 µl of a 5 mg/ml MTT solution was added to each well, and the plate was incubated in the dark at 37°C for 3 to 4 hours. Following this, the MTT solution was removed, and 100 µl of DMSO was added to each well to dissolve the purple crystals, which were then measured at 490 nm.

3. Results

Table 1. Cytotoxicity activity at different concentrations compared to control and negative controls

Control	20	40	60	80	100	120	140	160	180	200	Negative control
0.958	0.859	0.725	0.725	0.658	0.575	0.498	0.475	0.389	0.289	0.205	0.015
0.99	0.857	0.798	0.715	0.645	0.568	0.515	0.465	0.387	0.278	0.215	0.018
0.989	0.869	0.789	0.705	0.666	0.55	0.508	0.456	0.375	0.265	0.214	0.02

Table 1 shows the in-vitro cytotoxicity activity of the vitamin E-infused nanogel was evaluated at various concentrations (20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 µg/mL) compared to a control and a negative

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control. The results demonstrated a dose-dependent decrease in cell viability, indicating significant cytotoxic effects of the formulation on cancer cell lines.

At lower concentrations (20-60 µg/mL), a gradual decline in cell viability was observed, with OD values decreasing from **~0.859 to 0.725**. The cytotoxic effect became more pronounced at higher concentrations (80-200 µg/mL), where OD values significantly dropped to **~0.205** at 200 µg/mL. The highest cytotoxicity was observed at 200 µg/mL, with results close to the negative control, which had minimal OD values (~0.015-0.02).

Table 2. Cytotoxicity of the vitamin E-infused nano gel was evaluated using varying concentrations on oral cancer cell line

	Vitamin E infused Nanogel		
Control	97.81553	101.1442	101.0402
20	87.51734	87.30929	88.55756
40	82.52427	81.17198	80.23578
60	73.57836	72.53814	71.49792
80	66.60888	65.25659	67.44105
100	57.97503	57.24688	55.37448
120	49.96533	51.7337	51.00555
140	47.57282	46.53259	45.59639
160	38.62691	38.41886	37.1706
180	28.22469	27.08044	25.72816
200	19.48682	20.52705	20.42302

Table 2 shows the cytotoxicity of the vitamin E-infused nanogel was evaluated using varying concentrations (20–200 µg/mL) on cancer cell lines, and the results demonstrated a dose-dependent reduction in cell viability. At lower concentrations (20 µg/mL), the nanogel showed a moderate cytotoxic effect with approximately **87-88% cell viability** across replicates. As the concentration increased to 40 µg/mL, viability reduced to around **81-83%**, and further to **65-73%** at 60 µg/mL. A notable reduction in viability was observed at 100 µg/mL, where cell viability ranged between **55-58%**, nearing the IC50 value of **120 µg/Ml.**

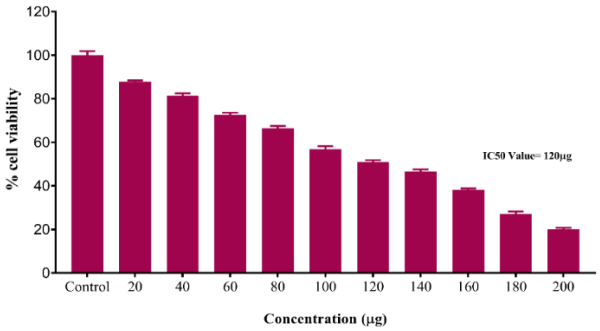

Figure 1. Cell viability at different concentrations of vit E nanogel formulation

Figure 1 shows the percentage of cell viability at different concentrations. A steep decline in viability was evident at concentrations beyond 120 µg/mL, with values reducing to 45-51% at 140 µg/mL and reaching a minimal viability of 19-22% at the highest concentration of 200 µg/where the percentage cell viability decreased significantly as the nano gel concentration increased.

IC50 Determination

The IC50 value, identified as **120 µg/mL**, signifies the concentration required to inhibit 50% of cell viability, showing it as the threshold for effective cytotoxic activity.

The dose-dependent cytotoxicity observed highlights the potential of vitamin E-infused nano gel as an anticancer therapeutic. The high efficacy at concentrations ≥120 µg/mL and consistency across replicates suggest that this formulation can selectively target cancer cells while maintaining its stability and activity. Further studies, including in-vivo models, are recommended to validate these findings and explore their clinical applicability. These findings suggest that the vitamin E-infused nanogel exhibits strong cytotoxic potential, especially at higher concentrations, effectively targeting cancer cells. The negative control confirmed the absence of external interference, validating the assay results. This dose-dependent activity underscores the formulation's potential as a therapeutic agent for cancer treatment, warranting further research.

4. Discussion

Vitamin E (VE) and its derivatives have been previously studied for their potential anticancer effects, with mixed results. While VE itself has shown little direct impact on

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cancer, its synthetic form, α-tocopheryl succinate (α-TOS), stands out as a promising candidate in cancer treatment [7]. The key difference between the two lies in how they work; Vitamin E acts as a redox-active antioxidant but doesn't trigger cell death in cancer cells, whereas α-TOS effectively promotes apoptosis, primarily by destabilizing mitochondria and altering cell signaling pathways related to apoptosis [8].

The limited effectiveness of Vitamin E in cancer prevention or treatment may stem from the body's tightly controlled levels of this vitamin. The liver keeps the amount of VE in circulation within a specific range, which means that even with supplements, it doesn't reach levels that would significantly impact cancer cells. Additionally, Vitamin E is quickly broken down and excreted, limiting its therapeutic potential [9]. On the other hand, α-TOS overcomes these hurdles by targeting cancer cells more directly and inducing cell death through methods that VE cannot. This includes its ability to interact with certain signaling molecules and destabilize mitochondria due to its unique structure [10].

Previous esearch has consistently shown that α-TOS outperforms Vitamin E in terms of suppressing tumor growth and killing cancer cells. In various animal studies, α-TOS has proven effective against several types of cancer, including breast cancer, melanoma, and colon cancer. Its effectiveness is attributed to its dual action: it not only encourages cancer cell death but also helps prevent the growth of new blood vessels that tumors need to thrive [11,12].

One of the reasons α-TOS is so selective for cancer cells is its structure. Cancer cells struggle to convert α-TOS into α-tocopherol (α-TOH), which is more common in healthy cells. This characteristic adds to its selective toxicity. Moreover, the slightly acidic nature of α-TOS allows it to be taken up more easily in the acidic environments typical of tumors, enhancing its anticancer capabilities. This suggests that there may be opportunities to design new VE analogues with improved effectiveness by tweaking their structures [13].

In addition to its standalone benefits, α-TOS works well in combination with other therapies, such as TRAIL (TNF-related apoptosis-inducing ligand). By engaging different pathways for triggering cell death, this combination can enhance the overall effectiveness and also tackle resistance that cancer cells might have. α-TOS can even make cancer cells more sensitive to TRAIL by inhibiting certain protective pathways, which opens new avenues for treatment strategies [14].

However, the journey toward using α-TOS and similar analogues in clinical settings is not without its challenges. One of the significant hurdles for compounds like α-TOS is that they tend to break down during digestion, which can make them hard to use orally and often require intravenous delivery. Fortunately, new developments, like ether analogues that resist breakdown, have paved the way for oral intake, although these changes might affect how well they work in the body. Striking a balance between efficacy, stability, and convenience remains key to making these treatments more viable.

The way α-TOS is processed in the body also offers some unique benefits. After it gets into the bloodstream, α-TOS binds with lipoproteins to ensure it reaches tumors effectively. Once it has done its work, α-TOS is converted in the liver back into α-TOH, which can re-enter circulation and act as an antioxidant. This dual function as both a cancer-fighting agent and a precursor to a well-known antioxidant highlights the diverse potential of α-TOS in cancer therapy.

The present study has shown that this novel vitamin E-infused nanogel formulation has shown a good cytotoxic activity against the oral cancer cell line so this formulation can be used in the OPMDs because of the formulations advantages.

5. Limitations and Future Scope

This research is confined to laboratory conditions, which may not entirely mimic the complex interactions present in the human body. To confirm the observed effects in a more comprehensive biological context, further research is crucial. Additionally, the potential for contamination during the formulation process could lead to undesirable outcomes. Future research should focus on biochemical analysis of the characterization of formulation. Furthermore, conducting ex-vivo experiments and clinical trials will be vital for evaluating the clinical efficacy of the vitamin E-infused nano gel formulation in OPMDs.

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6. Conclusion

This study demonstrates that a vitamin E-infused nanogel formulation exhibits enhanced cytotoxic activity against oral cancer cell lines. Formulated with vitamin E and cost-effective, biodegradable chitosan nanoparticles, this nano gel ensures targeted drug delivery. It also improves patient adherence and minimizes side effects. The nano gel shows potential as an alternative therapeutic option for oral potentially malignant disorders and mucocutaneous conditions, such as oral lichen planus and pemphigus vulgaris, offering improved therapeutic efficacy. Compared to traditional systemic oral vitamin E supplements, this nanogel provides a more effective and targeted approach. Future research should focus on in-vivo studies and clinical trials to further validate its therapeutic potential.

Ethical Approval: The Institutional research and ethical committee of Saveetha Dental College and Hospitals, Chennai, waived ethical clearance.

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Conflicts of interest: The authors declare that no conflicts of interest are associated with this work.

Author contributions: Amani T- conceptualization, data curation, formal analysis, investigation, methodology, and writing (original draft); Surenthar M- conceptualization, supervision, validation, visualization, and writing (review and editing)

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