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Protective Effects of Liposomal Vitamin C on SARS-CoV-2 Target Viral Entry Genes in Renal Cells

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Abstract

Background: The kidneys are a potential target for SARS-CoV-2 infection. Ascorbic acid (vitamin C) has been shown to play an important role in reducing the symptoms of SARS-CoV-2. Recently liposomal drug delivery platforms have demonstrated promising results in enhancing the effectiveness of various therapeutics including infectious diseases. In this study, we designed a liposomal delivery system containing vitamin C to evaluate its antiviral efficacy in COVID-19, focusing on its effects on viral entry gene expression in Vero cells.

Methods: Vitamin C was loaded into a liposome made up of hydrogenated soybean phosphatidylcholine, cholesterol, and 1,2-distearoyl-sn-glycero-3 phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000], and their physicochemical properties were assessed. Next, the cytotoxicity of free and liposomal vitamin C on the survival of the Vero cell line was evaluated using the MTT assay. In addition, the expression of viral entry genes, *angiotensin-converting enzyme 2* (ACE2) and *transmembrane protease serine 2* (TMPRSS2), key mediators of SARS-CoV-2 entry into kidney cells, was investigated using RTq-PCR.

Results: Liposomes were successfully loaded with vitamin C, achieving an encapsulation efficiency of 88.03%. The liposomal vitamin C formulation exhibited a brilliant surface morphology as observed by SEM. Both free and liposomal forms of vitamin C showed cytotoxic effects at higher concentrations. Moreover, both forms downregulated the expression of viral entry genes, although the liposomal form showed superior inhibitory performance compared to the free form.

***Conclusion*:** The study suggests liposomal vitamin C as a safe, effective treatment for COVID-19 by targeting viral entry genes in kidney cells, protecting them from viral damage and inflammation.

Keywords: Ascorbic Acid, COVID-19, Liposomes, Renal Cells, Viral Entry.

Introduction

Coronaviruses (CoVs) are a group of positive-strand RNA viruses with an envelope, among which Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused the coronavirus disease 2019 (COVID-19) pandemic, resulting in significant global morbidity and mortality (1, 2). Although COVID-19 is primarily a respiratory disease, the kidneys can also be among the target organs of infection by SARS-CoV-2, leading to acute kidney injury (AKI), particularly in patients with pre-existing kidney disease (3, 4), as previous studies have demonstrated the link between infections and AKI (5).

SARS-CoV-2 consists of four structural proteins: Spike (S), Envelope (E), Nucleocapsid (N), and Membrane (M). The S protein facilitates viral entry into host cells by

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interacting with angiotensin-converting enzyme 2 (ACE2). Transmembrane protease serine 2 (TMPRSS2) cleaves the spike protein, enabling its binding to ACE2 and promoting membrane fusion, a critical step in viral invasion (6). ACE2 is a component of the renin-angiotensin system (RAS), which maintains body homeostasis through two axes: Angiotensin-Converting Enzyme/Angiotensin II/ Angiotensin Type 1 Receptor (ACE/Ang Ⅱ /AT1R) as classical RAS, and Angiotensin-Converting Enzyme 2/Angiotensin 1-7/ Mas Receptor (ACE2/Ang1-7/ MasR) as the alternative RAS (7).

SARS-CoV-2 binding to ACE2 disrupts this balance, leading to inflammation, vascular damage, and multi-organ dysfunction, including acute kidney injury (AKI) (8, 9). Kidney involvement in COVID-19 has gained significant attention, as studies show that *ACE2* and *TMPRSS2* are highly expressed in renal cells, particularly in the proximal tubules, making the kidneys a critical target for SARS-CoV-2 (9). Direct viral invasion, systemic inflammation, and hypoxia all contribute to renal dysfunction in COVID-19 patients (10). Therefore, the modulation of *ACE2* and *TMPRSS2* expression in kidney cells could play a vital role in reducing SARS-CoV-2 infectivity and mitigating renal damage.

Ascorbic acid (vitamin C) has emerged as a promising candidate for COVID-19 therapy due to its potent antioxidant, anti-inflammatory, and immunomodulatory properties (11). Numerous studies have shown that vitamin C plays an important role in preventing and reducing the severity of many types of viral and bacterial infections (12). For example, giving vitamin C to septic mice with acute respiratory distress syndrome (ARDS) strengthened the epithelial barrier and improved alveolar fluid clearance by reducing the expression of inflammation-related genes (13, 14). Furthermore, vitamin C deficiency is directly associated with increased influenza A-induced lung pathology in mice (15). Arvinte et al. reported low vitamin C levels among critically ill COVID-19 patients admitted to intensive care units (ICUs) in the US (16). Another study also reported that low levels of vitamin C were associated with acute respiratory distress syndrome in COVID-19 patients (17). Additionally, vitamin C has been shown to reduce interleukin-7 (IL-7)–induced ACE2 expression in endothelial cells, suggesting a potential mechanism for limiting SARS-CoV-2 entry (18).

Despite its potential, the bioavailability of vitamin C is limited due to rapid metabolism and degradation in the digestive system (19). Liposomal systems enhance vitamin C stability, absorption, and controlled release, addressing its bioavailability limitations (20). Liposomes, composed of lipid bilayers, are particularly effective at delivering hydrophilic compounds like vitamin C, protecting them from degradation and improving therapeutic efficacy (21). Studies have demonstrated the advantages of liposomal vitamin C, including increased plasma concentrations without gastrointestinal side effects, which enables its use at higher therapeutic doses (22, 23).

This study aims to evaluate the therapeutic potential of free and liposomal vitamin C in targeting kidney cells, focusing on the modulation of *ACE2* and *TMPRSS2* to mitigate SARS-CoV-2-induced kidney damage.

Materials and Methods

Reagents

The Vero cell line (the epithelial kidney tissue normal cells, ATCC CCL-81™) was purchased from the Iranian Biological Resource Center (IBRC). MTT and vitamin C were obtained from Sigma-Aldrich (USA) and additional consumables including chloroform were prepared from Merck (Germany). Trypsin-EDTA, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from BioIdea (Iran), and DSPE-PEG2000, HSPC, cholesterol, and a dialysis membrane were purchased from Avanti Polar Lipids (USA). RNX-Plus Kit and PCR primers, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ACE2, and TMPRSS2 were purchased from Cinnagen (Iran) and SinaColon (Iran) respectively. cDNA synthesis kit and qRT-PCR SYBR green

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master mix were purchased from Yekta Tajhiz Arma (Iran) and Amplicon (Denmark) respectively.

Liposome Preparation

The synthesis of liposomes was carried out using a modified version of the standard thin film hydration technique (24). Initially, a lipid mixture comprising hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy (polyethylene glycol)-2000] (DSPE-PEG2000) was disolved in ethanol and chloroform at a 3:7 (v/v) ratio. The molar ratios of the lipids were set at 70:25:5, respectively. A total of 4.5 mg of vitamin C was added to this lipid solution in a round-bottom flask. The solvent was evaporated at 49 °C to achieve a thin lipid film. The resulting lipid film was thoroughly dried and rehydrated with 3 ml of phosphate-buffered saline (PBS) at pH 7.4, then manually shaken to ensure the film detached from the flask wall and dissolved uniformly. The rehydrated liposomal suspension was then homogenized at 3000 rpm for 5 minutes at 25 °C to reduce the particle size. Following homogenization, the liposomes underwent probe sonication at 75% amplitude in an ice compartment for 10 minutes to further refine their size and uniformity. To ensure sterility and achieve the desired size distribution, using sterile mixed cellulose ester (MCE) filters (0.22 μm), the liposomal suspension was filtered seven times (Sigma-Aldrich, USA). Finally, the liposomes were subjected to freeze-drying in a lyophilizer for 48 hours to obtain stable liposomal powders.

Determination of Encapsulation Efficiency (EE%)

Liposomes were separated from non-entrapped vitamin C through ultracentrifugation. The concentration of free vitamin C in the supernatant was determined using a spectrophotometer at a wavelength of 295 nm. A standard curve was used to quantify the vitamin C present in the supernatant. The calibration curve exhibited a high coefficient of determination ($R^2 = 0.999$).

Encapsulation efficiency was then calculated using Equation provided below.

$$\%EE = \frac{Amount\ of\ vitamin\ C\ used - amount\ of\ free\ vitamin\ C}{Amount\ of\ vtamin\ C\ used} \times 100$$

Characterization of Liposomes

Fourier-Transform Infrared Spectroscopy (FTIR)

A FTIR spectrometer (FTIR, Bruker Tensor, USA) was employed to analyze the functional groups of free vitamin C, empty liposomes, and vitamin C-loaded liposomes. The FTIR spectra were recorded for both vitamin C and liposomal formulations and across the wavenumber range of 400 to 4000 cm^{-1}.

Morphology Characterization

The morphology of the liposomes was analyzed through scanning electron microscopy (SEM). For sample preparation, the liposomes were freeze-dried to obtain a powdered form. The lyophilized powders were subsequently examined using an SEM instrument (SU660, Hitachi, Japan).

Kinetics of Vitamin C Release

Various kinetic models are commonly employed to describe the drug release process. In this study, various models, including zero-order, first-order, Higuchi, Hixson-Crowell, and Peppas, were employed to analyze the release kinetics of vitamin C from liposomes.

In zero-order systems, the drug is released at a constant rate, regardless of the concentration. The first-order model describes release in which the rate depends on the drug concentration. Higuchi's model explains release via Fickian diffusion, showing a linear relationship with the square root of time. Hixson-Crowell's model accounts for changes in particle size and surface area. The Peppas model categorizes release into four types: Fickian, case II, non-Fickian, and super case II, based on the release exponent. The percentage of drugs released versus log time follows a linear pattern in the Peppas model (25, 26).

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Cell Culture

The Vero cell line was used in this study due to its high expression of *ACE2* and *TMPRSS2* genes. In addition, many studies have shown that Vero cells have a high viral titer (27)**.** The Vero cell line was grown in plastic flasks ($75cm^2$ and $25cm^2$) using DMEM high glucose supplemented with 10% FBS and 1% antibiotic (penicillin-streptomycin) and incubated at 37 $^{\circ}C$, with 95% humidity and 5% CO_2.

Cell Viability Assay

After reaching 80-90% confluence, the cells were trypsinized and seeded into a 96-well microplate. After 24 h, untreated cell samples were used as the positive control. Cell treatment was performed at diverse concentrations of 100, 250, 500, 1000, and 10000 µM of free and liposomal forms of vitamin C for 24 h. MTT test was performed to evaluate the toxicity and find the best dose of free and liposomal forms of vitamin C on Vero cells. After replacing the culture medium of the treated cells with 100 µl of MTT solution, it was incubated (4h, 37 °C). Then, after removing the MTT solution, the formed formazan crystals were dissolved in 200 µl of dimethyl sulfoxide (DMSO). Eventually, absorbance was determined at 570 nm by an Elx800 ELISA microplate reader (USA).

Real time quantitative PCR (RTq-PCR)

A 12-well plate (25×10^4 cells/well) was used to seed Vero cells and incubate at 37 $^{\circ}C$. After 24 h, according to the results of the MTT test, the concentration of 1000 µM was utilized for cell treatment. The RNA was extracted using the Total RNA Extraction Mini Kit after 48 h. The RNA yield was determined using a NanoDrop spectrophotometer (Epoch, BioTek, USA). Then, reverse transcription was carried out on the extracted RNA using the cDNA synthesis kit. For real-time PCR, RTq-PCR SYBR green master mix and Real-Time PCR Machine (Rotor-Gene 6000, Qiagen, USA) were utilized. GAPDH was used as an internal control (Table 1). Amplification was done based on the following temperature program: First step: 95 $^{\circ}C$ for 3min, then 45 cycles and 40 cycles including 15 s at 95 $^{\circ}C$, 15 s at 54 $^{\circ}C$ and 15 s at 72° C for *ACE2* and *TMPRSS2*. The process was done in three stages and the relative expression of genes was calculated by the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

The difference between the treatment groups was determined using one-way ANOVA. Duncan's multiple range test was employed to measure significant differences between data groups using SPSS v20 and GraphPad Prism 9 ($P< 0.05$).

Results

Characterization of Liposomes Physically and Chemically

SEM imaging was employed to observe the morphology and structure of liposomes, including both empty and vitamin C-loaded variants. The SEM images revealed that both types of liposomes had a generally spherical shape and a consistent appearance (Fig. 1). Additionally, the liposomes loaded with vitamin C displayed greater brightness in comparison to the empty liposomes.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was used to examine the interactions between components by observing changes in vibration frequencies. The FTIR spectrum of empty liposomes (Figure 2) showed aromatic rings at 800-1000 cm^{-1}, C=C stretching at 1600 cm^{-1}, C-H groups between 2500-2900 cm^{-1}, and OH groups between 3300-3700 cm^{-1}. The spectrum of pure L-Ascorbic Acid revealed peaks at 1674 cm^{-1} (C=C) and 1322 cm^{-1} (enol-hydroxyl). After encapsulation, new peaks appeared at 3311.88 cm^{-1}, 1635.01 cm^{-1}, 1567.45 cm^{-1}, and 1377.59 cm^{-1}. These peaks correspond to the hydroxyl, band due to the scissor bending vibration of molecular water, acidic asymmetric stretch and C-H deformations of –CH2 or –CH3 groups (lignin) in aliphatic respectively. Following the encapsulation of vitamin C, a shift towards higher wavenumbers (3405 cm^{-1}) was observed in the

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position of this peak. This shift indicated the involvement of hydrogen bonding, particularly in the hydroxyl groups. This suggests hydrogen bonding plays a key role in vitamin C encapsulation into liposomes.

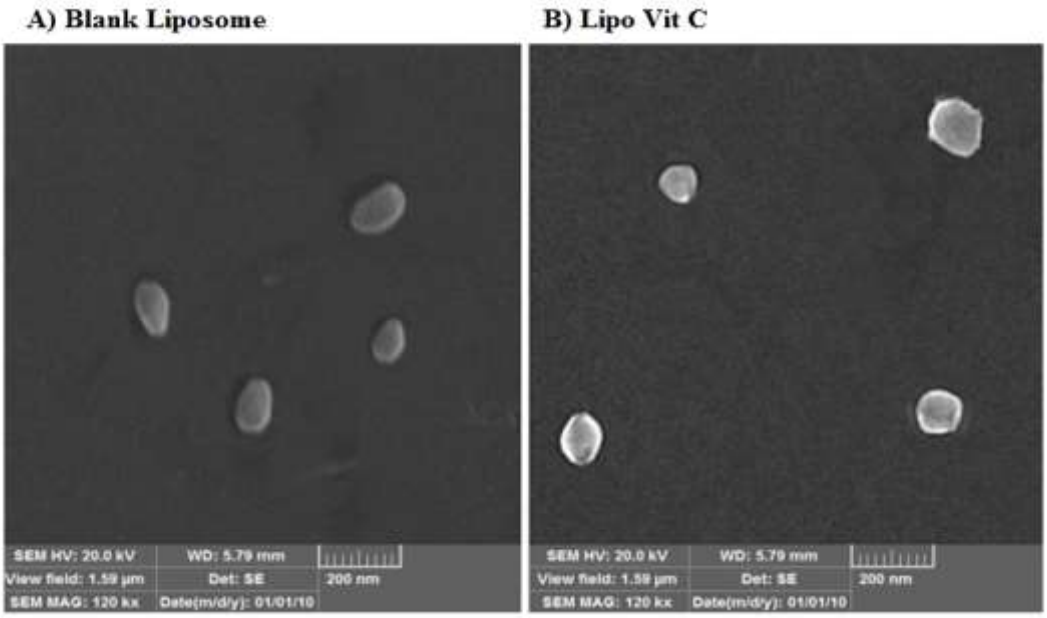

Fig. 1. The SEM images at magnifications of 1.59 µm. (A) Blank liposomes and (B) Vitamin C-loaded liposomes. The Vitamin C-loaded liposomes appear brighter than the blank ones, which indicate successful encapsulation and differences in surface characteristics due to the presence of Vitamin C.

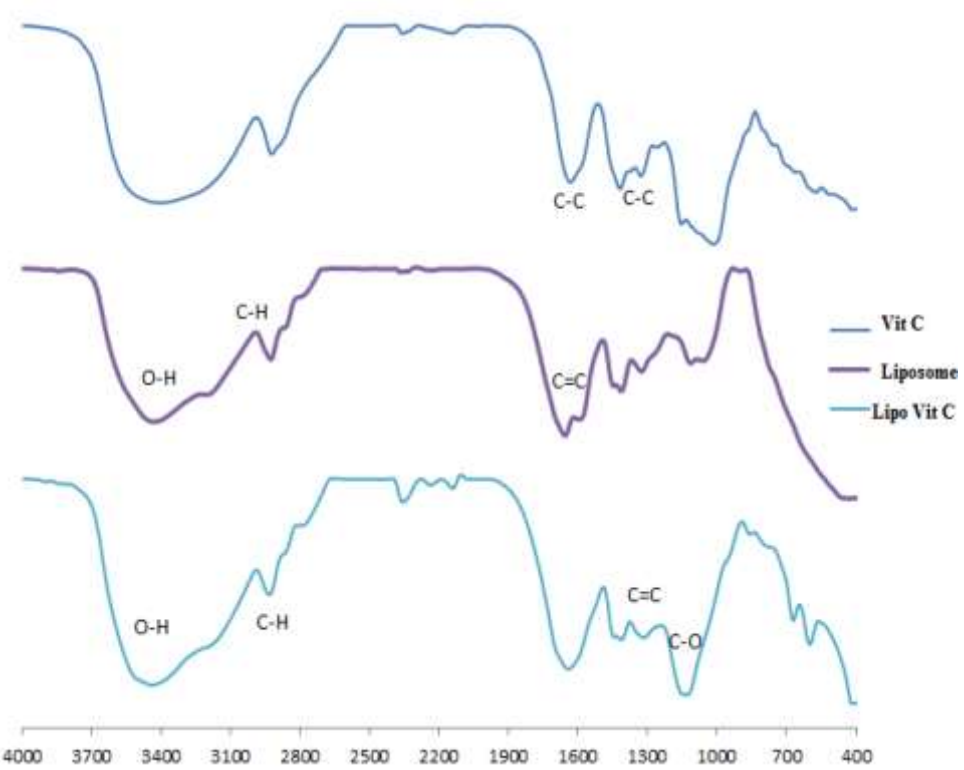

Fig. 2. Comparative FTIR spectrum of Vitamin C, Liposome, and Liposomal Vitamin C. The results show changes in vibrational peaks after encapsulation, indicating interactions between components and the role of hydrogen bonding in the encapsulation process.

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Vitamin C Release from Liposomes and Modeling

At pH 7.4 and over various time points (0.5 to 24 h), cumulative vitamin C release from liposomes was measured. Using the standard curve from encapsulation efficiency, the release was monitored. The release increased gradually during the first 8 h and then plateaued. The maximum release was approximately 93% (Fig. 3). Kinetic studies identified the Pepass model as the best fit for the release process at pH 7.4 (Table 2).

Table 1. The sequences of RTq-PCR primers.

Genes	Primer sequence	Refseq mRNA Accession
ACE2	F:5′- GCCTCCTCTCCTACTTTG -3′ R:5′- CTCAGCCCATCTTCTTCC -3′	**NM_021804**
TMPRSS2	F:5′- TGGGAAGTTTCAAATCAGC -3′ R:5′- GCATTCTTGGACGAGGG -3′	**NM_005656**
GAPDH	F:5′-CAATGACCCCTTCATTGACC-3′ R:5′-TGGAAGATGGTGATGGGATT-3′	**NM_002046.7**

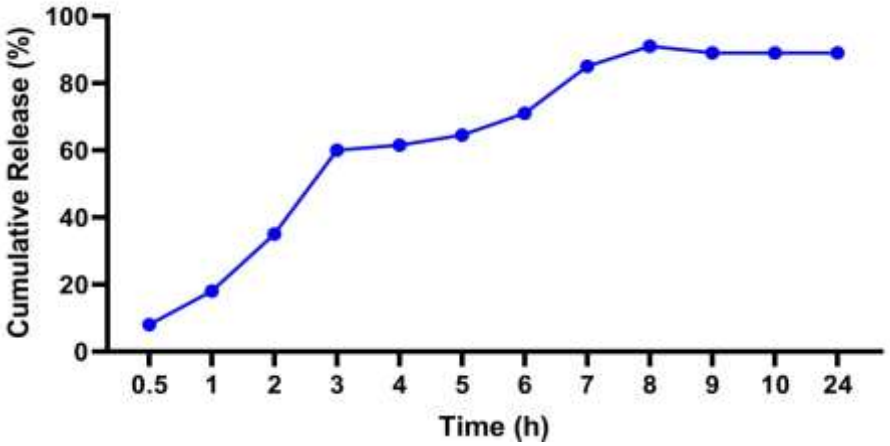

Fig. 3. Vitamin C release pattern from liposomal form, for 24 h and pH 7.4. The release gradually increased within the first 8 h and then plateaued. The maximum release reached approximately 93%, with the Peppas model providing the best kinetic fit.

Table 2. Kinetic Analysis of Vitamin C Release from Liposomes at pH 7.4.

pH=7/4	factor	Kinetic Models
8.33	K0	**Zero order**
0. 743	R^2	
28246.3	Ss	
-0.083	K_1	**One order**
0. 883	R^2	
46.03	Ss	
31.47	K_H	**Higuchi**
0.956	R^2	
4392.5	Ss	
1/24	K_P	**Peppas**
0/967	R^2	
2.51	Ss	
0.607	N	
-0.138	K_C	**Hixone Corel**
0.952	R^2	
50.11	Ss	

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Cell Viability Assay

Vero cells were treated with various concentrations of 100, 250, 500, 1000, and 10000 µM of free and liposomal forms of vitamin C. Our findings showed that the cytotoxic effects of vitamin C (free and liposomal) at high concentrations (in 1000 to 10000 µM) increased within 24 h (Fig. 4). In addition, the IC50 of the free and liposomal forms of vitamin C were calculated at 3477 µM and 2700 µM, respectively.

ACE2 and TMPRSS2 Gene Expression

The RTq-PCR method was used to investigate the expression changes of *ACE2* and *TMPRSS2*. The concentration of 1000 µM of the free and liposomal forms of vitamin C was chosen for RTq-PCR analyses. Free and liposomal forms of vitamin C decreased the expression of *ACE2* and *TMPRSS2* at the mRNA levels liposomal form of vitamin C was more successful (Fig. 5).

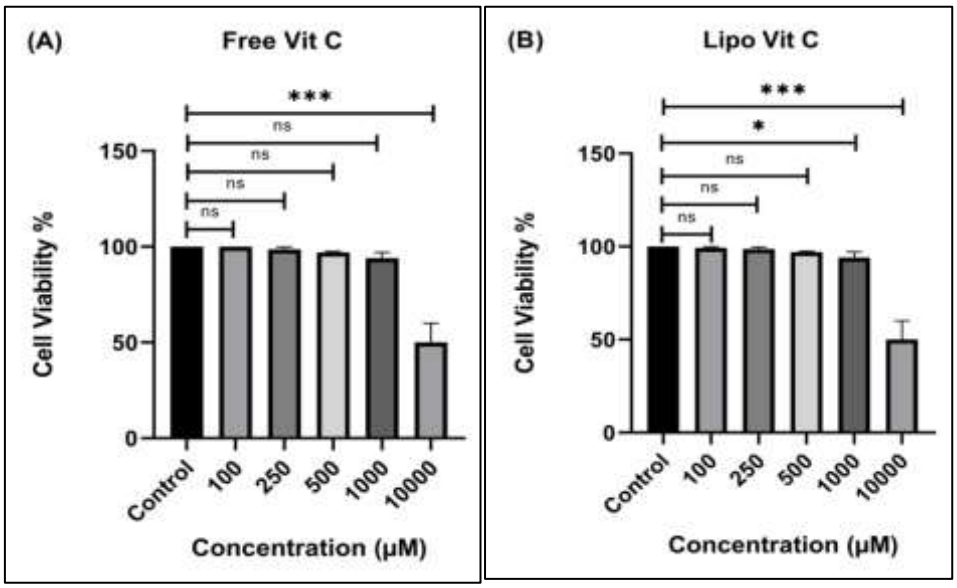

Fig. 4. The results of MTT assay in treatment with free form and liposomal (A) Free vitamin C, and (B) Liposomal vitamin C, for 24h. High concentrations (1000 to 10000 µM) of both forms exhibited cytotoxicity. A dose-dependent cytotoxicity was observed, with a lower IC50 in the liposomal form, P-value<0.05*/ P-value<0.001***.

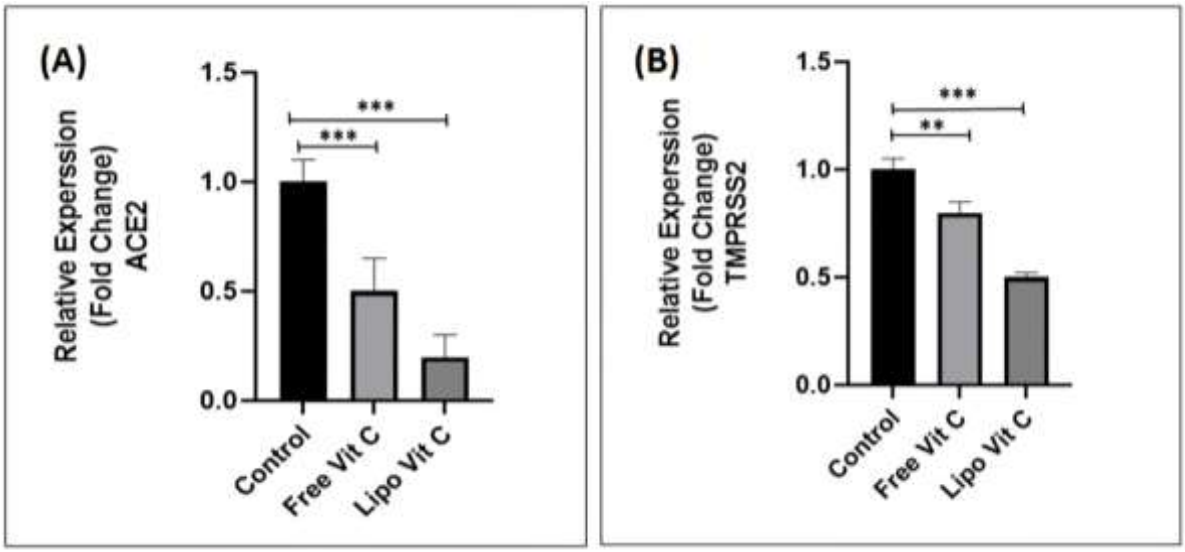

Fig. 5. The effect of vitamin C on the mRNA expression of A) *ACE2*, and B) *TMPRSS2* genes on Vero cell line in 1000 µM of concentration. Both free and liposomal forms of vitamin C reduced the expression of ACE2 and TMPRSS2, with the liposomal form showing a more significant effect. (P-value<0.01**/ P-value<0.001***).

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Discussion

This study explored the potential of liposomal vitamin C as a therapeutic agent to mitigate SARS-CoV-2 infection, particularly by modulating the expression of *ACE2* and *TMPRSS2* genes in kidney Vero cells. These two genes play a crucial role in viral entry into host cells, and their downregulation may reduce the susceptibility of cells to viral invasion (28). The results from this study not only support previous findings but also provide new insights into the effectiveness of liposomal vitamin C in targeting the kidney as a desirable site for SARS-CoV-2 infection.

We initially developed and characterized a liposomal structure containing vitamin C. The SEM and FTIR results confirmed the morphology of the liposomes and significant hydrogen bonding interactions, indicating successful encapsulation of vitamin C. Moreover, the sustained release profile of vitamin C from liposomes, achieving 93% release after 24 h, at pH 7.4, suggests that liposomes can provide a controlled release of vitamin C, a crucial factor for maximizing its therapeutic effects while minimizing side effects. These findings align with previous studies demonstrating that liposomal formulations can enhance the stability and bioavailability of encapsulated compounds such as vitamin C (19, 29). The controlled release of vitamin C is particularly important in the context of SARS-CoV-2 treatment, as vitamin C has been shown to possess antiviral, anti-inflammatory, and immune-modulatory properties (30). It appears that the liposomal system can prolong the presence of vitamin C in the bloodstream, thereby enhancing its antiviral efficacy and immune-modulating effects.

In the following, Cytotoxicity assays revealed that free and liposomal vitamin C reduced cell viability at high concentrations, with the liposomal formulation exhibiting a slightly lower IC50 (2700 μM compared to 3477 μM for the free form). This suggests that liposomal vitamin C can achieve similar therapeutic effects at lower concentrations, minimizing the risk of toxicity and gastrointestinal side effects. It has been reported that high doses of vitamin C are associated with gastrointestinal side effects (31). Our findings are consistent with the study showing that liposomal formulations reduce the toxicity of high-dose compounds by improving their solubility and bioavailability, thus reducing side effects and systemic toxicity (32).

While high doses of vitamin C have shown promise in preventing and treating viral infections, they are often associated with gastrointestinal disturbances and contraindications in individuals with kidney impairments or hemochromatosis (33, 34). With its enhanced bioavailability, liposomal vitamin C offers a potential solution by delivering therapeutic doses at lower concentrations, minimizing these side effects and providing a safer alternative for patients with underlying conditions.

Next, we examined the expression of *ACE2* and *TMPRSS2* genes using the RTq-PCR method and the most compelling result of this study was the downregulation of *ACE2* and *TMPRSS2* gene expression following treatment with both free and liposomal vitamin C. ACE2 serves as the primary receptor for SARS-CoV-2, while TMPRSS2 is crucial for the cleavage of the viral spike protein, a step necessary for viral entry (35). Given that ACE2 receptors are abundantly expressed on renal tubular cells, the kidneys have been increasingly recognized as major targets of SARS-CoV-2. COVID-19-induced AKI is a common complication, and studies have shown that *ACE2* and *TMPRSS2* are implicated in the pathogenesis of renal damage during SARS-CoV-2 infection (36, 37).

By downregulating *ACE2* expression, liposomal vitamin C may limit the virus's ability to enter and replicate within kidney cells, potentially reducing the incidence of COVID-19-associated AKI. Our findings align with studies showing that vitamin C can modulate *ACE2* expression, thus providing protection against viral entry (38). Furthermore, vitamin C has been shown to

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promote *ACE2* degradation, thereby reducing viral load and mitigating the severity of infection (39).

Interestingly, liposomal formulations have been shown to enhance the intracellular delivery of therapeutic agents, resulting in more pronounced biological effects compared to free drug formulations (40, 41). This is in agreement with our study, where liposomal vitamin C exhibited a more significant downregulation of *ACE2* and *TMPRSS2* compared to the free form in kidney cells.

In conclusion, our findings support the use of liposomal vitamin C as a potentially effective treatment for reducing SARS-CoV-2 infection by modulating *ACE2* and *TMPRSS2* expression. The enhanced bioavailability, controlled release, and lower toxicity profile of liposomal vitamin C make it a promising therapeutic option for COVID-19, particularly for patients with renal dysfunction.

While this study provides promising *in vitro* evidence of the potential benefits of liposomal vitamin C in reducing SARS-CoV-2 infection in kidney cells, further in vivo studies are needed to validate these findings in animal models and human trials. Given the critical role of ACE2 in SARS-CoV-2 entry and the high expression of *ACE2* in kidney cells, liposomal vitamin C could offer a novel therapeutic approach for preventing or treating COVID-19-associated renal complications. Additionally, clinical trials are necessary to determine the optimal dosing and safety profiles of liposomal vitamin C in COVID-19 patients, particularly those with pre-existing kidney dysfunction.

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Conflict of Interest

All the authors have declared no conflicts of interest.

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References

1. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W,W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Erratum in: Nature. 2020;588(7836):E6.
2. Rostami-Far Z, Rahmani K, Mansouri K, Khadem Erfan MB, Shaveisi-Zadeh F, Nikkhoo B. Genetic Regulation of Interleukin-6 and Interleukin-10 in COVID-19 Infection. Rep Biochem Mol Biol. 2023;12(2):284-293.
3. Liakopoulos V, Roumeliotis S, Papachristou S, Papanas N. COVID-19 and the kidney: time to take a closer look. Int Urol Nephrol. 2022;54(5):1053-1057.
4. Rai V. COVID-19 and Kidney: The Importance of Follow-Up and Long-Term Screening. Life (Basel, Switzerland). 2023;13(11):2137.
5. Kahar LA. Interleukin-6 and Procalcitonin as Potential Predictors of Acute Kidney Injury Occurrence in Patients with Sepsis. Rep Biochem Mol Biol. 2024;13(2):144-153.
6. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nature Reviews Molecular Cell Biology. 2022;23(1):3-20.
7. Musavi H, Abazari O, Barartabar Z, Kalaki-Jouybari F, Hemmati-Dinarvand M, Esmaeili P, et al. The benefits of Vitamin D in the COVID-19 pandemic: biochemical and immunological mechanisms. Arch Physiol Biochem. 2023;129(2):354-62.
8. Mizuiri S, Ohashi Y. ACE and ACE2 in kidney disease. World J Nephrol. 2015;4(1):74-82.
9. Soleimani M. Acute Kidney Injury in SARS-CoV-2 Infection: Direct Effect of Virus on

[DOI: 10.61882/rbmb.13.4.484]

Kidney Proximal Tubule Cells. Int J Mol Sci. 2020;21(9):3275.
10. Ahmadian E, Hosseiniyan Khatibi SM, Razi Soofiyani S, Abediazar S, Shoja MM, Ardalan M, Zununi Vahed S. Covid-19 and kidney injury: Pathophysiology and molecular mechanisms. Rev Med Virol. 2021;31(3):e2176.
11. Holford P, Carr AC, Jovic TH, Ali SR, Whitaker IS, Marik PE, Smith AD. Vitamin C-An Adjunctive Therapy for Respiratory Infection, Sepsis and COVID-19. Nutrients. 2020;12(12):3760.
12. Hemilä H, de Man AME. Vitamin C and COVID-19. Front Med (Lausanne). 2021;7:559811.
13. Fisher BJ, Seropian IM, Kraskauskas D, Thakkar JN, Voelkel NF, Fowler AA 3rd, Natarajan R. Ascorbic acid attenuates lipopolysaccharide-induced acute lung injury. Crit Care Med. 2011 Jun;39(6):1454-60.
14. Fisher BJ, Kraskauskas D, Martin EJ, Farkas D, Wegelin JA, Brophy D, et al. Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid. Am J Physiol Lung Cell Mol Physiol. 2012;303(1):L20-32.
15. Li W, Maeda N, Beck MA. Vitamin C deficiency increases the lung pathology of influenza virus-infected gulo-/- mice. J Nutr. 2006;136(10):2611-6.
16. Arvinte C, Singh M, Marik PE. Serum Levels of Vitamin C and Vitamin D in a Cohort of Critically Ill COVID-19 Patients of a North American Community Hospital Intensive Care Unit in May 2020: A Pilot Study. Med Drug Discov. 2020;8:100064.
17. Chiscano-Camón L, Ruiz-Rodriguez JC, Ruiz-Sanmartin A, Roca O, Ferrer R. Vitamin C levels in patients with SARS-CoV-2-associated acute respiratory distress syndrome. Crit Care. 2020;24(1):522.
18. Ma S, Sun S, Li J, Fan Y, Qu J, Sun L, et al. Single-cell transcriptomic atlas of primate cardiopulmonary aging. Cell Res. 2021;31(4):415-32.
19. Purpura M, Jäger R, Godavarthi A, Bhaskarachar D, Tinsley GM. Liposomal delivery enhances absorption of vitamin C into plasma and leukocytes: a double-blind, placebo-controlled, randomized trial. Eur J Nutr. 2024;63(8):3037-3046.
20. Khuntawee W, Amornloetwattana R, Vongsangnak W, Namdee K, Yata T, Karttunen M, Wong-Ekkabut J. *In silico* and *in vitro* design of cordycepin encapsulation in liposomes for colon cancer treatment. RSC Adv. 2021;11(15):8475-8484.
21. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A. 1996;93(8):3704-9.
22. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A. 1996;93(8):3704-9.
23. Prantl L, Eigenberger A, Gehmert S, Haerteis S, Aung T, Rachel R, et al. Enhanced Resorption of Liposomal Packed Vitamin C Monitored by Ultrasound. J Clin Med. 2020;9(6):1616.
24. Sabaghi Y, PourFarzad F, Zolghadr L, Bahrami A, Shojazadeh T, Farasat A, Gheibi N. A nano-liposomal carrier containing p-coumaric acid for induction of targeted apoptosis on melanoma cells and kinetic modeling. Biochem Biophys Res Commun. 2024;690:149219.
25. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67(3):217-23.
26. Paarakh MP, Jose PA, Setty C, Peter Christoper. Int J Pharm Res Technol. Release kinetics–concepts and applications. 2018;8(1):12-20.
27. Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, et al. Isolation and characterization of SARS-CoV-2 from the first US COVID-19 patient. bioRxiv [Preprint]. 2020:2020.03.02.972935.
28. Gkogkou E, Barnasas G, Vougas K, Trougakos IP. Expression profiling meta-analysis of ACE2 and TMPRSS2, the putative anti-inflammatory receptor and priming protease of SARS-CoV-2 in human cells, and

[DOI: 10.61882/rbmb.13.4.484]

identification of putative modulators. Redox Biol. 2020;36:101615.
29. Pamunuwa GK, Karunaratne DNJJoFQ. Liposomal Delivery of Plant Bioactives Enhances Potency in Food Systems: A Review. J Food Quality. 2022;2022(1):5272592.
30. Rs N, Reddy MVNJ, Batra S, Srivastava SK, Syal K. Vitamin C and its therapeutic potential in the management of COVID19. Clin Nutr ESPEN. 2022;50:8-14.
31. Wang Y, Mackenzie B, Tsukaguchi H, Weremowicz S, Morton CC, Hediger MA. Human vitamin C (L-ascorbic acid) transporter SVCT1. Biochem Biophys Res Commun. 2000;267(2):488-94.
32. Liu P, Chen G, Zhang JJM. A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. Molecules. 2022;27(4):1372.
33. Tavill AS; American Association for the Study of Liver Diseases; American College of Gastroenterology; American Gastroenterological Association. Diagnosis and management of hemochromatosis. Hepatology. 2001;33(5):1321-8.
34. Thomas LD, Elinder CG, Tiselius HG, Wolk A, Akesson A. Ascorbic acid supplements and kidney stone incidence among men: a prospective study. JAMA Intern Med. 2013;173(5):386-8.
35. Gkogkou E, Barnasas G, Vougas K, Trougakos IP. Expression profiling meta-analysis of ACE2 and TMPRSS2, the putative anti-inflammatory receptor and priming protease of SARS-CoV-2 in human cells, and identification of putative modulators. Redox Biol. 2020;36:101615.
36. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. Intensive Care Med. 2020;46(6):1114-1116.
37. Simões E Silva AC, Teixeira MM. ACE inhibition, ACE2 and angiotensin-(1-7) axis in kidney and cardiac inflammation and fibrosis. Pharmacol Res. 2016;107:154-162.
38. Ivanov V, Goc A, Ivanova S, Niedzwiecki A, Rath M. Inhibition of ACE2 Expression by Ascorbic Acid Alone and its Combinations with Other Natural Compounds. Infect Dis (Auckl). 2021;14:1178633721994605.
39. Zuo Y, Zheng Z, Huang Y, He J, Zang L, Ren T, et al. Vitamin C promotes ACE2 degradation and protects against SARS-CoV-2 infection. EMBO reports. 2023;24(4):e56374.
40. Reus P, Guthmann H, Uhlig N, Agbaria M, Issmail L, Eberlein V, et al. Drug repurposing for the treatment of COVID-19: Targeting nafamostat to the lungs by a liposomal delivery system. J Control Release. 2023;364:654-671.
41. Que H, Hong W, Lan T, Zeng H, Chen L, Wan D, et al. Tripterin liposome relieves severe acute respiratory syndrome as a potent COVID-19 treatment. Signal Transduct Target Ther. 2022;7(1):399.

[DOI: 10.61882/rbmb.13.4.484]