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A New Sterilization Strategy Using TiO₂ Nanotubes for Production of Free Radicals that Eliminate Viruses and Application of a Treatment Strategy to Combat Infections Caused by Emerging SARS-CoV-2 during the COVID-19 Pandemic

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Abstract: Synthesized titanium oxide nanoparticles (TiO₂-NPs) nanotubes were used for the disinfection of new emerging corona virus-19 (SARS-CoV-2) in this study. The newly synthesized TiO2-NPs (nanotubes) were characterized by chemical spectroscopic analysis Fourier-transform infrared spectroscopy and ultraviolet FT-IR and UV. The chemical purity and Zeta potential distribution of the TiO₂-NPs (nanotubes) were evaluated to confirm their nano-range, and their surface morphology was determined by scanning electron microscopy (SEM), transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HR-TEM), and energy dispersive X-ray analysis (EDX). The antiviral activity of the TiO2-NPs (nanotubes) against SARS-CoV-2 was evaluated using 10% (Dimethyl sulfoxide) DMSO and dist.H2O using a cytotoxicity assay and inhibitory concentration assay (to determine the cytotoxic half concentration CC_{50} and half maximal inhibitory concentration IC₅₀). The current results confirmed that TiO₂-NPs exhibit strong anti-SARS-CoV-2 activity at very low cytotoxic concentrations in vitro with a non-significant selectivity index $(CC_{50}/IC_{50} \le 1)$. The obtained results indicate that TiO₂-NPs and nanotubes have potent antiviral activity at a very low concentrations (IC₅₀ = 568.6 ng/mL), with a weak cytotoxic effect on the cellular host ($CC_{50} = 399.1 \text{ ng/mL}$). Thus, we highly recommend the use of TiO₂-NPs (nanotubes) in vitro and in wall coatings as a potent disinfectant to combat SARS-CoV-2 with little irritation of the cellular hosts. Furthermore, we also recommend more and excessive prospective studies on the complexation of natural active or natural compounds with TiO₂-NPs (nanotubes) to minimize their cytotoxicity, enhance their antiviral activity, and increase their inhibition of SARS-CoV-2.

Keywords: titanium oxide nanoparticles; COVID-19; SARS-CoV-2; HRTEM; SEM; TEM; cytotoxicity; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide MTT assay; antiviral activity

1. Introduction

SARS-CoV-2 is considered a Ribonucleic acid "RNA" virus. RNA viruses cause numerous diseases, particularly in mammals, and are considered major pathogens in many diseases including Hepatitis C Virus (HCV), Ebola disease, SARS, influenza, measles and retrovirus, and human immunodeficiency virus (HIV) [1]. CoV infections first affect the upper respiratory tract of mammals. Coughing and fever are considered major symptoms of CoV infection in humans, and breathing difficulty is also evident as a result of SARS-CoV-2. COVID-19's clinical symptoms include severe respiratory disorders caused by SARS-CoV-2, such as excessive inflammation and oxidation [2].

Viruses are small proteins containing genetic information. In the case of enveloped viruses, viruses can spread via two essential routes: by direct cell contact or through the aqueous environment [3].

SARS viruses appeared globally in 2002 and 2003. About 8000 people were infected by SARS-CoV, resulting in about 800 deaths. The effects of SARS-CoV-2 differ from those of other types of coronaviruses, and SARS-CoV-2 is spreading widely in humans. The SARS-CoV-2 situation is considered to be a pandemic, and the virus has infected millions of patients in the past year [4].

Emerging coronaviruses have drawn major global concern [5]. SARS-CoV-2 viruses are generally spherical, with a diameter varying between 70 and 140 nm, and the surfaces of SARS-CoV-2 viruses are covered in spikes [6]. SARS-CoV-2 can be transmitted from infected persons by the exhalation of SARS-CoV-2 particles, which then attach to surfaces that are touched by receiving persons [5].

Titanium oxide nanostructures (TiO₂-NPs) have potent antibacterial and antiviral properties, in addition to the ability to be used as drug carriers, and are widely used in modern medicine [6,7]. In animal production, these NPs are useful as an alternative to antibiotics for combating antibiotic-resistant bacteria and as growth promoters, the application and consumption of which are targeted for reduction in many countries. Additionally, metal NPs are applied to nutrient delivery, increasing meat production, increasing milk and egg quality, and increasing the quality of sperm [8].

TiO₂-NPs are widely used nanomaterials in a large number of applications, including painting, skincare, and cosmetic products [9]. The global output of TiO₂-NPs is expected to be 2.5 million tons/year by 2025 [10]. This widespread use of TiO₂-NPs leads to their accumulation and high release in aquatic environments [10].

Although TiO₂-NPs at very low concentrations are speculated to be non-toxic, toxicity has been confirmed at high doses. TiO₂-NPs have a strong ability to absorb other toxicants due to their large surface area. Exposure to TiO₂-NPs (nanostructures) is reported to inhibit growth and cause oxidative stress, injury, and impairment in some animals [9].

The potency of nanoparticles as a new class of antibacterial and antiviral drug has been considered; however, new studies of nanoparticles for combating bacterial pathogens, and Gram-positive and Gram-negative bacteria, are needed [10–12]. Greener nanoparticle assay combinations with standard drugs are of recent interest to clarify antimicrobial resistance of free toxic ions [13–15]. Various metal oxide nanoparticles are also of importance in studies; in particular, TiO₂-NPs (nanostructures) are considered to be safe substance by the United States Food and Drug Administration USFDA, and considered to be an element for the synthesis of nucleic acid and hemopoiesis in tissues of the body.

The current study was designed to evaluate the antiviral activity of synthesized TiO₂-NPs against SARS-CoV-2 by the chemical characterization of TiO₂ nanoparticles. We aimed to assess the use of TiO₂-NPs (nanostructures) as a sterilization compound against SARS-CoV-2 infection, estimate their cytotoxicity, and review prospective studies to elevate their antiviral activity.

2. Materials and Methods

2.1. Ethical Approval

The biological antiviral activity of TiO₂-NPs (nanotubes) was performed at the center of Scientific Excellence for Influenza Viruses, National Research Centre, 12311 Dokki, Giza on (SARS-CoV-2) (hCoV-19/Egypt/NRC-03/2020 (Accession Number on GSAID: EPI_ISL_430820). Graphical abstract for the experimental protocol and summarization of the study as shown in (Scheme 1).



Scheme 1. Graphical abstract for the experimental protocol and summarization of the study.

2.2. Synthesis of Titanium Oxide Nanostructure

TiO₂-NPs were synthesized according to [16] in two steps. In the first, 0.2 M TiO₂ was synthesized by adding 0.80 g of TiO₂ to 100 mL of H₂O dist. and stirring for 2 h. Another solution with 10.32 g of citrate of tri-sodium (50 mL H_2O) was synthesized and added dropwise to the first solution. This rate was maintained for about 2 h. Then, a precipitate was produced in the solution; the precipitate was washed 3 times before centrifuging for 15 min. The precipitate was then dried in an oven at 1100 °C for about 24 h, yielding TiO_2 -NP powder. A quantity of 20 mL HNO₃ solution was then mixed with the remaining samples and sonicated for 1 h, before the addition of 0.1 M of sodium tri citrate followed by 2 h sonication. The synthesized samples were then washed 4 times. TNTs were prepared using a hydrothermal process described in [17]. Then, 5 g of TiO₂-NPs prepared by the sol-gel method were mixed with 500 mL of a 10 M NaOH aqueous solution, followed by hydrothermal treatment at 150 °C (TNTs) in a Teflon-lined autoclave for 12 h. After the hydrothermal reaction, the treated powders were washed thoroughly with distilled water and 0.1 M HCl, and subsequently filtered and dried at 80 °C for 1 day. To achieve the desired TNT size and crystallinity, the powders were calcined in air at 500 °C for 1 h [16,17]. The resulting TiO₂-NPs were used to testing their activity against SARS-CoV-2.

2.3. Chemical Characterization of TiO₂-Nanostructures

By using tools of analysis, the shape and size of TiO₂-Nps were characterized using images of scanning electron microscopy (Tokyo, Japan), transmition electron microscopy (Tokyo, Japan) and high resolution transmition electron microscopy (Nakagyo-ku, Kyoto, Japan). Ti and O analysis measured using EDX. Electronic spectrum of (Nakagyo-ku, Kyoto, Japan) TiO₂-nanostructure was analyzed using UV-Vis spectroscopy (Nakagyo-ku, Kyoto, Japan). The IR analysis performed mixing TiO₂-Nanostrucure powder with KBr. The X-ray diffraction patterns were recorded on X'Pert PRO PAN-analytical X-ray powder diffraction (Nakagyo-ku, Kyoto, Japan), target copper with secondary monochromate.

2.4. Zeta Potential Measurements

It is a technique for assessment of the particle surface charge in the solution, which is directly related to the stability of the nanoparticle suspension. Zeta potential values revealed the stability of the prepared nanoparticles and nanotubes. The pH effect on the size of particles and the zeta potential distributions of TiO_2 -Nps (nanostructures) were evaluated by using pH from 5 to 12 by drop wise addition of solutions of 0.1 M NaOH or HCl.

2.5. Cytotoxicity Assay

To evaluate the cytotoxic half concentration (CC₅₀) (maximal) of TiO₂-NPs, stock solutions of TiO₂-NPs in 10% concentration of DMSO were prepared in "dist.H₂O" and diluted to the solutions of Dulbecco's Modified Eagle Medium DMEM. Cytotoxic activity of TiO₂-NPs was evaluated and tested in VERO-E6 cells by using MTT assay. VERO-E6 cells were completely seeded in (3×10^5 cells/mL, about 100 µL/each well) concentration, then incubated for 24 h in CO₂ (5%) at 37 °C for (24 h) later, VERO-E6 cells, the host for SARS-CoV-2 were treated with different concentrations of TiO₂-NPs in triplicates. Then, the upper supernatants were completely discarded, and the monolayers were excessively washed with sterile (PBS) buffer phosphate saline for (3 times) and addition of (20 µL of TiO₂-NPs) of MTT solution to 96 well-plates and incubated for about 4 h at 37 °C. The formed crystals of formazan were dissolved with isopropanol treated with HCl. Their absorbance was measured at λ max 540 nm with 620 nm as a reference λ by using a multiwell plate reader (Nakagyo-ku, Kyoto, Japan). The % of cytotoxicity as compared to the untreated cells was determined by the following Equation (1).

The % cytotoxicity against sample conc. was used to calculate the conc. which exhibited 50% of the cellular cytotoxicity (CC_{50}).

% cytotoxicity = $\frac{(absorbance of cells without treatment - absorbance of cells with treatment) \times 100}{absorbance of cells without treatment}$ (1)

2.6. Determination of Inhibitory Concentration 50% (IC₅₀)

In plates (96-well) of the tissue cultures, 2.4×10^4 Vero-E6 cells were distributed in the wells and incubated overnight at a humidified 37 °C incubator under 5% CO₂. The cellular monolayers were then washed twice with PBS "1×" and subjected to virus adsorption (Accession Number: EPI_ISL_430820) for about 1 h at 37 °C. The cellular monolayers were overlaid with approximately 100 µL of DMEM which containing different concentrations of TiO₂-NPs. After the incubation in CO₂ incubator for about 72 h, VERO-E6 cells were treated with paraformaldehyde for about 20 min and stained with staining of crystal violet in dist.H₂O for about 15 min at 37 °C. A total of 100 µL methanol (100%) was used for each well for dissolving the crystal violet dye and the color optical intensity of TiO₂-NPs was measured at 570 nm by using plate reader (Anthos Zenyth 200rt, The Netherlands). IC₅₀ of TiO₂-NPs (nanotubes) is needed to decrease cytopathic effect of SARS-CoV-2-induced by about 50%, relative to control SRAS-CoV-2.

3. Results and Discussion

3.1. Infrared Spectrum of Synthesized TiO₂-NPs

The spectrum of infrared for TiO₂-NPs provides spectral peaks started from 551 to 419 cm⁻¹; where the strong bands at 433 and 419 cm⁻¹ can be attributed to the stretching vibration mode of Ti-O (Figure 1) [18].



Figure 1. FT-IR of synthesized TiO₂-NPs.

3.2. Electronic Spectrum of TiO₂-NPs

The UV–Vis absorption spectra of the synthesized TiO₂-NPs (nanostructures) yielded a broad absorption spectrum that appeared at a wavelength of 350 nm. This result is consistent with those in the literature [19]. Figure 2 shows the electronic spectrum of TiO₂-NPs. The electronic spectrum of TiO₂ is shown in Figure 3, after heating at 150 °C. The peak refers to titanium oxide produced, strong peak resulted at wavelength of 350 nm. This was due to the band intrinsic absorption gap for titanium oxide produced due to the electronic transition from the valence band to the conduction band [20]. The band gap energy (Eg) of titanium oxide is estimated according to Eg = hc/ λ , where c is the light speed, equal to 3 × 10⁸ m/s; h is the constant plank, equal to 6.626 × 10⁻³⁴ Js; and λ is the wavelength (m) [21,22]. The energy of band gap is equal to 3.26 eV.



Figure 2. UV spectra of TiO₂-NPs.





Size Distribution by Intensity



3.3. Zeta Potential and Size Distribution Intensity

The diameter of TiO₂-NPs (nanostructures) and Zeta potential in dist.H₂O at pH value of ~7.36 determined by ultrasonication using Dynamic light scattering DLS of prepared dispersions using 0.2 mg of TiO₂-NPs in 10 mL of H₂O deionized. TiO₂-NPs (nanostructures) particles' diameter average of TiO₂-NPs was measured to be 470.6 nm with zeta potential of -5.92 mV and Polydispersity index pdI = 0.364. Zeta potential and size of particles TiO₂-NPs were determined (Figure 3a,b) using pH 6–12 and the zeta potential decreases while the size of particles increases by increasing the pH. Measurements of TiO₂-NPs (nanostructures) surface area attributed to at various TiO₂-NPs (nanostructures) burning temperatures occurred by N₂-adsorption of N₂ at 77 K. As temperature of calcination elevated from (29 m²·g⁻¹) for 82 ± 10 nm TiO₂-NPs (nanostructures) at 100 °C to (7 m²·g⁻¹) for 265 ± 8 nm at 600 °C surface area of TiO₂-NPs (nanostructures) decreased which is convenient with a previous study [20].

3.4. Zeta Size Measurements

Table 1 shows the mean particle size or the average dynamic sizes of the TiO_2 -NPs (nanostructures) were found 953.6 d·nm with pdI equal to 0.526.

Table 1. Zeta potential and particles size analysis.

Aspect	PdI	Z-Average (d∙nm)	Zeta Potential (mV)
Value	0.526	953.6	-1.02

3.5. Scanning Electron Microscope (SEM)

Figure 4a,b represents a scanning electron microscopy (SEM) image of TiO₂-NPs (nanostructures). The morphology of the TiO₂-NPs (nanotubes) was examined, and the size of the grains was nearly uniform. The morphology surfaces of the prepared TiO₂-NPs (nanostructures) powders were measured using SEM analytical techniques, such as wide plates and nodes with homogenously sized distributions for TiO₂-NPs. Figure 4b shows an FE-SEM image of the sample anatase TiO₂-NPs nanotubes, which were grown at 150 °C for 12 h, and exhibit a pure tube-like structure. The length of the TiO₂-NPs nanotubes is several micrometers, their diameter is approximately 50 to 100 nm, and they are very uniform, relatively clean, and smooth-surfaced. Figure 3 shows the surface morphology of the electrode film on the FTO glass. Chemical composition analysis by EDX confirmed the Ti and O peaks in the TiO₂-NPs (nanostructures). The sample elemental analysis resulted in values of 78% for Ti and 20% for O, which confirms the high purity of TiO₂-NPs (nanotubes) [21,22].





3.6. Transmission Electron Microscope (TEM) and High Resolution Transmittance Electron Micrographs (HRTEM) of TiO₂-NPs

The morphology of the TiO₂-NPs (nanostructures) sample was determined by using TEM. Figure 5a,b shows a typical TEM image of the TiO₂-NPs. The size and morphology of the TiO₂-NPs (nanostructures) were estimated using HRTEM micrographs. TiO₂-NP nanoparticles were found in the 40–60 nm range with a spherical to polygonal medium. HRTEM Figure 6a,b showed a lattice space of 0.22 for TiO₂ planes.



(d) 33.53nm 36.51nm 31.22nm 46.08nm 2.99nm 31.95nm 31.95nm 31.95nm

(b)

Figure 5. (**a**,**b**) Transmission Electron microscope (TEM) of titanium nanostructures TiO₂-NPs (100 and 200 nm).



Figure 6. (a,b) High resolution transmission electron microscope (HR-TEM) images of titanium nanostructures TiO_2 -NPs.

3.7. XRD of TiO₂-Nanotubes

XRD for TiO₂-NPs (nanostructures) which provides the sol-gel pattern at 450 °C, confirming the presence of anatase and rutile phases' mixture. Prominent anatase peaks and prominent rutile peaks of TiO₂-NPs (nanostructures) clearly presented using XRD pattern confirming the nanoparticle structure. The patterns of the TiO₂ nanotube films prepared at a hydrothermal temperature of 180 °C for 14 h are shown in Figure 7. By the hydrothermal method, TiO₂ nanoparticles were observed to be transformed into the anatase phase. From XRD patterns, the TiO₂-NPs (nanostructures) possess crystallized highly anatase structure with high degree of purity. The rutile peaks for TiO₂-NPs nanotubes, refers to complete transformation to anatase.



Figure 7. Titanium nanostructures (TiO₂-NPs) cytotoxicity and inhibatory concentration against emerging corona virus (SARS-CoV-2) activity. Graph of Cytotoxicity concentration 50 (CC₅₀): on Vero E6 cells. Graph of Inhibitory concentration 50 (IC₅₀): Antiviral activity against Severe Acute. Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (hCoV-19/Egypt/NRC-03/2020.

3.8. Cytotoxicity of TiO₂-NPs and Inhibition Activity against SARS-CoV-2

TiO₂-NPs showed potent antiviral activity against SARS-CoV-2 in very low concentration of 568.6 ng/mL. The MTT assay was used to evaluate potential cell cytotoxicity of TiO₂-NPs. The results showed that 399.1 ng/mL of TiO₂-NPs significantly reduced the viability of VERO-E6 cells (Figure 8).

3.9. Anti-Viral Activity and Cytotoxicity of Titanium Oxide Nanoparticles and Inhibition Activity Against SARS-CoV-2

TiO₂-NPs (nanotubes) showed potent antiviral activity against SARS-CoV-2 in very low concentration of 568.6 ng/mL, by using MTT assay to elucidate the potential cell cytotoxicity of TiO₂-NPs. The results showed that 399.1 ng/mL of TiO₂-NPs (nanostructures) significantly reduced the viability of VERO-E6 cells as shown in (Figure 8) and images of the host cells "Vero-E6" cell after the incubation with SARS-CoV-2 (after the stage of infection) which showed a high potent anti-viral activity of TiO₂-NPs against SARS-Cov-2, the cells' sheet that was treated with TiO₂-NPs showing enlarged holes and patches around SRAS-CoV-2 as shown in (Figure 8A–C).



Figure 8. (A–C) Images for Vero-E6 host cells (After infection) after the incubation with SARS-CoV-2 and treated with TiO₂-NPs which showed potent anti-viral activity of TiO₂-NPs against SARS–Cov-2, the sheet of cells treated with TiO₂-NPs showing high enlarged patches around SRAS-CoV-2 cells, thus demonstrating the ability of TiO₂-NPs (nanostructures) to disinfect SARS-CoV-2 and offer a high level of inhibition to its growth. Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (hCoV-19/Egypt/NRC-03/2020).

Differences between zinc oxide nanoparticles (ZnO-NPs) inhibition activity [23–27] on SARS-CoV-2 cells as cited in our previous work [28] and inhibition activity of titanium oxide nanoparticles and its nanotubes (TiO₂-NPs) as shown in Table 2 clarified that TiO₂-NPs has more potent antiviral activity against SARS-CoV-2 than ZnO-NPs, and showed a higher CC_{50}/IC_{50} ratio in TiO₂-NPs (0.7) than ZnO-NPS.

Table 2. Comparison between inhibition activity for SARS-CoV-2 (differences between antiviral activity of both nanoparticles against SARS-CoV-2).

Aspect	Zinc Oxide Nanoparticles	Titanium Oxide Nanoparticles	
CC ₅₀	292.2 ng/mL [28]	399.1 ng/mL	
IC ₅₀	526 ng/mL [28]	568.6 ng/mL	
CC_{50}/IC_{50} ratio	0.55	0.70	
Inhibition activity of SARS-CoV-2 (Antiviral activity)	ZnO-Nps < TiO ₂ -NPs (Titanium oxide nanoparticles possess antiviral activity in concentration 586.6 ng/mL more than Zinc oxide nanoparticles concentration 526 ng/mL, this means that ZnO-NPs induced anti-viral activity in concentration less than TiO ₂ -NPs but TiO ₂ -NPs induced less cytotoxicity than ZnO-NPs and this means that TiO ₂ -NPs has antiviral activity against SARS-CoV-2 and it is safer than ZnO-NPs with less cytotoxicity).		

4. Discussion

Viral prevention remains a major objective for the maintenance of human health, and the essential concept is the inhibition of viral replication. Current antivirals have been weakened due to the elevation of drug resistance, particularly during the COVID-19 pandemic [23]. Thus, the release of new effective anti-COVID-19 treatments is urgently needed.

Nanoparticles provide significant benefits because they are highly effective as antiviral agents, even in low concentrations. In addition, nanoparticles have vital characteristics that ensure their suitability for different types of coating [24–27].

The present study reported that TiO₂-NPs (nanostructures) have potent anti-SARS-CoV-2 activity with very low cytotoxicity, as indicated by their SI value (IC₅₀/CC₅₀ \leq 1). This finding is consistent with [16], who revealed that TiO₂-NPs (nanostructures) have stronger antiviral activity and low cytotoxicity when coated with Polyethylene Glycated -NPs, compared to TiO₂-NPs alone. Results of the current study thus highlight the use of TiO₂-NPs as a disinfecting agent against SARS-CoV-2 with low cytotoxicity. Similarly, we suggest using TiO₂-NPs (nanostructures) coated with Polyethylene Glycated (polyethylene Glycated), which plays a major role in enhancing and elevating the antiviral activity against SARS-CoV-2 virus, and may reduce the cellular cytotoxicity of TiO₂-NPs (nanostructures) for the host cells.

Additional confirmation of our findings and suggestion to coat TiO_2-NPs (nanotubes) was provided in our previous research [28]. A recent explanation is that TiO_2-NPs produce Ti^{+2} ions and thus produce a large quantity of reactive oxygen species (ROS); these free radicals can damage proteins, lipids, carbohydrates, and DNA, and eventually lead to cellular apoptosis [29,30]. Therefore, we suggest coating TiO_2-NPs (nanostructures) with polyethylene glycol or natural active compounds that can provide strong anti-SARS-CoV-2 activity to reduce cytotoxicity and prevent the release of reactive oxygen by the masking of TiO_2-NPs (nanostructures).

Nanoscience has recently identified an essential vital role of nanoparticle agents due to their direct inhibition of a wide range of microbes. Nanoparticles such as TiO₂-NPs (nanotubes) are considered to be effective agents for carrying a large number of drugs and vaccines. Additionally, the size of nanomaterials accelerates their drug delivery involvement due to their surface functions. As a result, nanoparticles have the capability to co-transport drugs and numerous agents [31].

The relationship between SARS-CoV-2A and this vital aspect of nanomaterials is evidence of the competitive ability of the host with a viral binding surface cellular receptor because receptors of ACE2 are essential in the action of SARS-CoV-2 entering host cells [32]. Hence, blocking of ACE2 receptors could help in fighting SARS-CoV-2 [33]. Therefore, preventing SARS-CoV-2 infection in host cells plays an important role in combatting viral infection. To prevent viruses entering the human body, it is important to prepare nano-formulas using recently developed nanotechnology to prevent viral entry via ACE2 receptors [34]. Additionally, the catalytic activity and stability of ACE can be enhanced using nanoparticles to inhibit entry of SARS-CoV-2 [34,35]. Therefore, if TiO₂-NPs (nanotubes) are used in coatings for hospitals walls, the spread of SARS-CoV-2 may be significantly reduced. Thus, we proposed a hypothetical antiviral mechanism for TiO₂-NPs [36–39], including ACE2, which is able to block the SARS-CoV-2 entry into the cellular host by inhibiting its attachment to the ACE2 receptors [40].

The current finding broadly supports our previous work [28], which revealed that metal nanoparticles, ZnO-NPs, demonstrated significant activity against different types of microorganisms, including most viruses. The reported mechanism was the same as that shown in the current study; however, TiO₂-NPs revealed more antiviral activity with less cytotoxicity than ZnO-NPs.

As indicated in previous studies [17], TiO_2 -NPs (nanostructures) have attracted increasing attention as an antimicrobial agent. TiO_2 -NPs (nanostructures) have the ability to bind to the bacterial cellular wall and can enter the cellular membrane via a significant

number of modifications, resulting in bacterial cellular death [41]. Another mechanism is the development of "pits" at the cellular surface, resulting in the accumulation of TiO_2 -NPs (nanostructures) [42], in turn leading to the accumulation of free radicals that cause cellular death [43,44]. Due to the global COVID-19 pandemic, the transmission of the SARS-CoV-2 virus largely occurs by direct viral attachment to cellular hosts. TiO_2 will be of significant benefit by inducing the triggering of free radicals on the viral cell surface and thus causing the death of the virus. This concept confirms our findings relating to the anti-SARS-CoV-2 and inhibition activities in very low concentrations, and is considered to be a promising result.

The results of the current study are consistent with the findings of [45], who demonstrated that TiO_2 -NPs (nanostructures) have a large number of potential applications due to their high chemical stability, wide photoactivity, high pH range, and the presence of numerous active adsorption sites on their surface, which may help in absorbing pollutants. Due to their high photoactivity, TiO_2 -NPs (nanostructures) are widely applied to the removal of water pollutants in water treatment.

TiO₂ has a wide range of antimicrobial activity, as shown in several studies using TiO₂ against different types of microorganisms, such as viruses [46,47]. The efficiency of TiO₂-NPs (nanotubes) is related to their surface reactions, surface morphology, and surface size ratio [48]. Researchers have focused on TiO₂ synthesis with new features that make it suitable for a large number of green environmental applications, such as the effective control of viral infections. Negatively charged TiO₂-NPs (nanostructures) can block viruses and inhibit their attachment to host cells.

It was somewhat unexpected to obtain data that confirm the anti-COVID-19 activity of TiO_2 -NPs (nanotubes) with low cytotoxicity. These findings verify the anti-SARS-CoV-2 activity of TiO_2 -NPs at a low concentration (568.6 ng/mL) with low cytotoxicity, which can be alleviated by coating TiO_2 -NPs (nanotubes) with natural active compounds or polymeric compounds, such as PEGylated. These coatings may reduce the diameter of the particles and moderate the triggering of cytotoxic effects of ROS on the cellular host, thus achieving effective anti-SARS-CoV-2 action in low concentrations. These findings reveal the potential for the use of TiO_2 -NPs (nanostructures) to combat COVID-19.

The use of metal oxides in nanotechnology represents a new strategy for combatting microorganisms, owing to direct antiviral activity or enhancing an organism's resistance to the pathogen. In this study, the ability of TiO_2 -NPs (nanostructures) to inhibit SARS-CoV-2 and, thus, their antiviral activity were tested. The results showed a significant decline in virus activity. In addition, our results also showed the high efficacy of TiO_2 -NPs nanotubes.

Previous studies showed that hepatic enzymes were elevated in experiments on male rats, indicating that damage had occurred in the plasma membrane structure due to the presence of reactive oxygen species (ROS) produced during the oxidative stress. These results are in agreement with El-Shenawy et al. [49] who found that the elevation of hepatic enzymes could be due to oxidative stress induced by TiO₂ nanostructures, which caused an imbalance in the permeability of the plasma membrane. Elevated levels of serum hepatic enzymes are indicative of cellular leakage and the loss of the functional integrity of cell membranes in tissues, which, in turn, signify tissue injury [49].

Different metal oxide nanotubes have been developed using different methodologies, precursors, and fibers. TiO_2 -NPs nanotubes have been obtained using different techniques [23–25]. However, no development has been carried out with antiviral purposes, particularly against the SARS-CoV-2 virus.

TiO₂ is one of the most widely applied nanomaterials and is extensively used in food applications [50]. TiO₂-NPs nanostructures have been previously developed with antimicrobial goals. TiO₂ nanotubes have been shown to reduce *E. coli* and *S. aureus*. Recently, Jian et al. developed TiO₂ and silver loaded-TiO₂ antibacterial agents using the sol-assay method with minimal inhibition concentration [51,52].

TiO₂-NPs (nanostructures) have been reported to induce the excessive production of free radicals and thus cause oxidative stress and a series of inflammatory processes and

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cytotoxicity [53]. These findings are in accordance with the current findings, which showed that prepared TiO_2 -NPs nanotubes induced excessive free radicals, resulting in oxidative stress that may induce cytotoxicity in the context of SARS-CoV-2.

Despite the promising properties of TiO_2 -NPs (nanostructures), research is underway to change the NPs' surfaces' characteristics in order to elevate the efficiency of reactive oxygen species generation and to improve its chemical properties. Surface-modified TiO₂-NPs can make it to be doped with various metal ions [54] or combined with various dyes [55,56] and this will very helpful for coatings of walls with this new synthesized nanotubes of TiO₂-NPs (nanostructures) that can give sterilization option for coatings. Surface complexes that enhancing TiO₂ as photosensitizers include transition metals. Inorganic ligands, such as CN^- , F^- , PO_4^{3-} can also link surface titanium. If the molecule absorbs a photon with energy higher than its value, it passes to an excited state and can trigger more electrons that are negatively charged in the conduction band, leaving positively charged holes in the valence band. Free \bar{e} may attack surrounding O₂ and H₂O molecules to form reactive oxygen species, including superoxide (O^{2-}) , (H_2O_2) , and hydroxyl radical (•OH) [57]. These forms of superoxides are highly unstable in the biological systems and react with the cellular components causing necrotic cell death or apoptotic. It has also been proven that TiO₂-NPs (nanostructures) inhibit bacterial multidrug resistance [36]. Human health threats from SARS viruses have been present over time. Uncontrolled bat, avian mouse and human SARS-CoV- dispersal can impact both global public health and economic stability [58]. All these finding confirmed the obtained results and recommend using synthesized (TiO₂-NPS) (nanostructures) as a sterilization agent against SARS-CoV-2.

5. Conclusions

TiO₂-NPs (nanostructures) were fully chemically characterized using different spectroscopic tools. The synthesized titanium nanotubes have a potent antiviral activity against SARS-CoV-2 at low concentration and can trigger many free radicals that may cause oxidative stress to SARS-CoV-2, which is a very promising result in combating SARS-CoV-2. Thus, we recommend more prospective studies applied to TiO₂-NPs immobilization with other active compounds that may reduce this cytotoxicity and enhance its anti-SARS-CoV-2 activity.

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References

- Perlman, S.; Netland, J. Coronaviruses post-SARS: Update on replication and pathogenesis. *Nat. Rev. Microbiol.* 2009, 7, 439–450.
 [CrossRef]
- 2. Zhang, B.; Zhou, X.; Zhu, C.; Feng, F.; Qiu, Y.; Feng, J.; Jia, Q.; Song, Q.; Zhu, B.; Wang, J. Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. *medRxiv* 2020, 7, 157.
- 3. Tsuneo, I. Anti-viral vaccine activity of zinc (II) for viral prevention, entry, replication, and spreading during pathogenesis process. *Curr. Trends Biomed. Eng. Biosci.* **2019**, *19*, 1–6.
- 4. Hamza, R.Z.; Al-Talhi, T.A.; Gobouri, A.; Al-Sanie, W.; El-Megharbel, S.M. Are favipiravir and acyclovir with IgG injections supplemented with vitamin D "suggested therapeutic option" can fight against COVID-19? *Adv. Anim. Vet. Sci.* **2021**, *9*, 549–554.
- 5. Jialiang, Z.; Zexu, H.; Fatemeh, Z.; Zhigang, C.; Meifang, Z. Progress and perspective of antiviral protective material. *Adv. Fiber Mater.* **2020**, *2*, 123–139.
- 6. Zhou, P.; Yang, X.L.; Wang, X.G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.R.; Zhu, Y.; Li, B.; Huang, C.L.; et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **2020**, *579*, 270–273. [CrossRef] [PubMed]
- Baranowska-Wójcik, E.; Szwajgier, D.; Oleszczuk, P.; Winiarska-Mieczan, A. Effects of titanium dioxide nanoparticles exposure on human health—A review. *Biol. Trace Elem. Res.* 2020, 193, 118–129. [CrossRef] [PubMed]
- 8. Hamza, R.Z.; Diab, A.A. Testicular protective and antioxidant effects of selenium nanoparticles on monosodium glutamateinduced testicular structure alterations in male mice. *Toxicol. Rep.* **2020**, *7*, 254–260. [CrossRef]
- Hussein, M.M.A.; Gad, E.; Ahmed, M.M.; Arisha, A.A.; Mahdy, H.F.; Swelum, A.A.A.; Tukur, H.A.; Saadeldin, I.M. Amelioration of titanium dioxide nanoparticle reprotoxicityby the antioxidants morin and rutin. *Environ. Sci. Pollut. Res.* 2019, 26, 29074–29084. [CrossRef]
- Cheng, H.; Yan, W.; Wu, Q.; Lu, J.; Liu, C.; Hung, T.C.; Li, G. Adverse reproductive performance in zebrafish with increased bioconcentration of microcystin- LR in the presence of titanium dioxide nanoparticles. *Environ. Sci. Nano.* 2018, *5*, 1208–1217. [CrossRef]
- 11. Ruddaraju, L.K.; Pammi, S.V.N.; Sankar, G.G.; Padavala, V.S.; Kolapalli, V.R.M. A review on anti-bacterials to combat resistance: From ancient era of plants and metals to present and future perspectives of green nano technological combinations. *Asian J. Pharm. Sci.* **2019**. [CrossRef] [PubMed]
- 12. Raghunath, A.; Perumal, E. Metal oxide nanoparticles as antimicrobial agents: A promise for the future. *Int. J. Antimicrob. Agents.* **2017**, *49*, 137–152. [CrossRef] [PubMed]
- 13. Wang, L.; Hu, C.; Shao, L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int. J. Nanomed.* **2017**, *12*, 1227–1249. [CrossRef] [PubMed]
- 14. Baker, S.; Pasha, A.; Satish, S. Biogenic nanoparticles bearing antibacterial activity and their synergistic effect with broad spectrum antibiotics: Emerging strategy to combat drug resistant pathogens. *Saudi Pharm. J.* **2017**, *25*, 44–51. [CrossRef]
- Fayaz, A.M.; Balaji, K.; Girilal, M.; Yadav, R.; Kalaichelvan, P.T.; Venketesan, R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. *Nanomed. Nanotechnol. Biol. Medicine.* 2010, *6*, 103–109. [CrossRef] [PubMed]
- 16. Sara, A.; Khuram, S.; Sadaf, M.; Iftikhar, A.; Muhammad, H.R.; Syed Muhammad, F. Antibacterial and antiviral potential of colloidal Titanium dioxide (TiO₂) nanoparticles suitable for biological applications. *Mater. Res. Express* **2019**, *6*, 105409.
- 17. Alexandros, B.; Tracy, De Peralta.; Richard, D.H. The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on Streptococcus mutans using a suite of bioassays. *Nanotoxicology* **2014**, *8*, 1–16.
- 18. Hoffmann, M.R.; Martin, S.T.; Choi, W.; Bahnemann, D.W. Environmental applications of semiconductor photocatalysis. *Chem. Rev.* **1995**, *95*, *69–96*. [CrossRef]
- 19. Bagheri, S.; Chandrappa, K.; Hamid, S.B.A. Facile synthesis of nano-sized ZnO by direct precipitation method. *Pharm. Chem.* **2013**, *5*, 265–270.
- 20. Hamza, R.Z.; Al-Salmi, F.A.; El-Shenawy, N.S. Zinc oxide nanoparticles with green tea extract complex in the pancreas of rats against monosodium glutamate toxicity. *J. Basic Clin. Physiol. Pharmacol.* **2020**. [CrossRef]
- 21. Bagabas, A.; Alshammari, A.; Aboud, M.F.; Kosslick, H. Room temperature synthesis of zinc oxide nanoparticles in different media and their application in cyanide photodegradation. *Nanoscale Res. Lett.* **2013**, *8*, 1–10. [CrossRef] [PubMed]
- 22. Nagarajan, S.; Kuppusamy, K.A. Extracellular synthesis of zinc oxide nanoparticle using seaweeds of gulf of Mannar. *India. J. Nanobiotechnol.* **2013**, *11*, 39. [CrossRef] [PubMed]
- 23. Salahuddin, N.A.; El-Kemary, M.; Ibrahim, E.M. Synthesis and characterization of ZnO nanoparticles via precipitation method: Effect of annealing temperature on particle size. *Nanosci. Nanotechnol.* **2015**, *5*, 82–88.
- 24. Makau, J.N.; Watanabe, K.; Mohammed, M.M.; Nishida, N. Antiviral activity of peanut (*Arachis hypogaea* L.) skin extract against human influenza viruses. *J. Med. Food* **2018**, *21*, 777–784. [CrossRef] [PubMed]
- 25. Tavakoli, A.; Ataei-Pirkooh, A.; Mm Sadeghi, G.; Bokharaei-Salim, F.; Sahrapour, P.; Kiani, S.J.; Moghoofei, M.; Farahmand, M.; Javanmard, D.; Monavari, S.H. Polyethylene glycol-coated zinc oxide nanoparticle: An efficient nanoweapon to fight against herpes simplex virus type 1. *Nanomedicine* **2018**, *13*, 2675–2690. [CrossRef] [PubMed]
- 26. Reed, L.J.; Muench, H.A. simple method of estimating fifty per cent endpoints. Am. J. Epidemiol. 1938, 27, 493–497. [CrossRef]

- Zhou, J.; Zhao, F.; Wang, Y.; Zhang, Y.; Yang, L. Size-controlled synthesis of ZnO nanoparticles and their photoluminescence properties. J. Lumin. 2007, 122, 195–197. [CrossRef]
- El-Megharbel, S.M.; Alsawat, M.; Al-Salmi, F.A.; Hamza, R.Z. Utilizing of (zinc oxide nano-spray) for disinfection against "SARS-CoV-2" and testing its biological effectiveness on some biochemical parameters during (COVID-19 pandemic)—"ZnO nanoparticles have antiviral activity against (SARS-CoV-2)". *Coatings* 2021, 11, 388. [CrossRef]
- 29. Ghaffari, H.; Tavakoli, A.; Abdolvahab, M.; Alijan, T.; Bokharaei-Salim, F.; Masoumeh, Z.; Mohammad, F.; Davod, J.; Seyed, J.K.; Esghaei, M.; et al. Inhibition of H1N1 influenza virus infection by zinc oxide nanoparticles: Another emerging application of nanomedicine. *J. Biomed. Sci.* **2019**, *26*, 1–10. [CrossRef]
- Siddiqi, K.S.; Rahman, A.; Husen, A. Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Res. Lett.* 2018, 13, 141. [CrossRef]
- Sangiliyandi, G.; Muhammad, Q.; Youngsok, C.; Jeong, T.D.; Chankyu, P.; Kwonho, H.; Jin-Hoi, K.; Hyuk, S. Antiviral potential of nanoparticles—Can nanoparticles fight against coronaviruses? *Nanomaterials* 2020, 10, 1645.
- 32. Verdecchia, P.; Angeli, F.; Reboldi, G. Angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers and coronavirus. *J. Hypertens.* **2020**, *5*, 1020–1026.
- Jessup, J.A.; Gallagher, P.E.; Averill, D.B.; Brosnihan, K.B.; Tallant, E.A.; Chappell, M.C.; Ferrario, C.M. Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. *Am. J. Physiol. Heart Circ. Physiol.* 2006, 291, 2166–2172. [CrossRef]
- 34. Paolo, V.; Claudio, C.; Antonio, S.; Fabio, A. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur. J. Intern. Med.* **2020**, *76*, 14–20.
- 35. Kenney, P.; Hilberg, O.; Laursen, A.C.; Peel, R.G.; Sigsgaard, T. Preventive effect of nasal filters on allergic rhinitis: A randomized, double-blind, placebo-controlled crossover park study. *J. Allergy Clin. Immunol.* **2015**, *136*, 1566–1572. [CrossRef]
- 36. Maness, P.C.; Smolinski, S.; Blake, D.M.; Huang, Z.; Wolfrum, E.J.; Jacoby, W.A. Bactericidal activity of photocatalytic TiO₂ reaction: Toward an understanding of its killing mechanism. *Appl. Environ. Microbiol.* **1999**, *65*, 4094–4098. [CrossRef]
- Kim, B.; Kim, D.; Cho, D.; Cho, S. Bactericidal effect of TiO₂ photocatalyst on selected food-borne pathogenic bacteria. *Chemosphere* 2003, 52, 277–281. [CrossRef]
- Hajipour, M.J.; Fromm, K.M.; Ashkarran, A.A.; de Aberasturi, D.J.; de Larramendi, I.R.; Rojo, T.; Serpooshan, V.; Parak, W.J.; Mahmoudi, M. Antibacterial properties of nanoparticles. *Trends Biotechnol.* 2012, *30*, 499–511. [CrossRef]
- 39. Fenoglio, I.; Greco, G.; Livraghi, S.; Fubini, B. Non- UV induced radical reactions at the surface of TiO₂ nanoparticles that may trigger toxic responses chemistry. *A Eur. J.* **2009**, *15*, 4614–4621. [CrossRef]
- Li, W.; Hulswit, R.J.G.; Kenney, S.P.; Widjaja, I.; Jung, K.; Alhamo, M.A.; van Dieren, B.; van Kuppeveld, F.J.M.; Saif, L.J.; Bosch, B.J. Broad receptor engagement of an emerging global coronavirus may potentiate its diverse cross-species transmissibility. *Proc. Natl. Acad. Sci. USA* 2018, 115, E5135–E5143. [CrossRef]
- 41. Imai, Y.; Kuba, K.; Rao, S.; Huan, Y.; Guo, F.; Guan, B.; Yang, P.; Sarao, R.; Wada, T.; Leong-Poi, H.; et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005, *436*, 112–116. [CrossRef]
- 42. Aydemir, D.; Ulusu, N.N. Correspondence: Angiotensin-converting enzyme 2 coated nanoparticles containing respiratory masks, chewing gums and nasal filters may be used for protection against COVID-19 infection. *Travel Med. Infect. Dis.* **2020**, *37*, 101697. [CrossRef]
- 43. Refat, M.S.; Hamza, R.Z.; Adam, A.M.A.; Saad, H.A.; Gobouri, A.A.; Al-Salmi, F.A.; Altalhi, T.A.; El-Megharbel, S.M. Potential Therapeutic Effects of New Ruthenium (III) Complex with Quercetin: Characterization, Structure, Gene Regulation, and Antitumor and Anti-Inflammatory Studies (RuIII/Q Novel Complex Is a Potent Immunoprotective Agent). *Crystals* **2021**, *11*, 367. [CrossRef]
- 44. Hamza, R.Z.; Al-Harbi, M.S.; Al-Hazaa, M.A. Neurological Alterations and Testicular Damages in Aging Induced by D-Galactose and Neuro and Testicular Protective Effects of Combinations of Chitosan Nanoparticles, Resveratrol and Quercetin in Male Mice. *Coatings* **2021**, *11*, 435. [CrossRef]
- 45. Elsharkawy, M.M.; Derbalah, A. Antiviral activity of titanium dioxide nanostructures as a control strategy for broad bean strain virus in faba bean. *Pest Manag. Sci.* 2018, 75, 828–834. [CrossRef] [PubMed]
- 46. Blake, D.M.; Maness, P.C.; Huang, Z.; Wolfrum, E.J.; Huang, J.; Jacoby, W.A. Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. *Sep. Purif. Rev.* **1999**, *28*, 1–50. [CrossRef]
- 47. Makowski, A.; Wardas, W. Photocatalytic degradation of toxins secreted to water by cyanobacteria and unicellular algae and photocatalytic degradation of the cells of selected microorganisms. *Curr. Top. Biophys.* **2001**, *25*, 19–25.
- Adán, C.; Marugán, J.; Sánchez, E.; Pablos, C.; Grieken, R. Understanding the effect of morphology on the photocatalytic activity of TiO₂ nanotube array electrodes. *Electrochim. Acta* 2016, 191, 521–529. [CrossRef]
- 49. El-Shenawy, N.S.; AL-Harbi, M.S.; Hamza, R.Z. Effect of vitamin E and selenium separately and in combination on biochemical, immunological and histological changes induced by sodium azide in male mice. *Exp. Toxicol. Pathol.* 2015, 67, 65–76. [CrossRef]
- 50. De dicastillo, C.L.; Patino, C.; Galotto, M.; Palma, J.L.; Alburquenque, D.; Escrig, J. Novel antimicrobial titanium dioxide nanotubes obtained through a combination of atomic layer deposition and electrospinning technologies. *Nanomaterials* **2018**, *8*, 128. [CrossRef] [PubMed]
- 51. Jiang, X.; Lv, B.; Wang, Y.; Shen, Q.; Wang, X. Bactericidal mechanisms and effector targets of TiO₂ and Ag-TiO₂ against Staphylococcus aureus. *J. Med. Microbiol.* **2017**, *66*, 440. [CrossRef]

- 52. Dudefoi, W.; Moniz, K.; Allen-Vercoe, E.; Ropers, M.-H.; Walker, V.K. Impact of food grade and nano-TiO₂ particles on a human intestinal community. *Food Chem. Toxicol.* **2017**, *106*, 242–249. [CrossRef] [PubMed]
- 53. Reeves, J.F.; Davies, S.J.; Dodd, N.J.F.; Jha, A.N. Hydroxyl radicals (•OH) are associated with titanium dioxide (TiO₂) nanoparticleinduced cytotoxicity and oxidative DNA damage in fish cells. *Mutat. Res.* **2008**, *640*, 113–122. [CrossRef]
- 54. Ziental, D.; Czarczynska-Goslinska, B.; Mlynarczyk, D.T.; Glowacka-Sobotta, A.; Stanisz, B.; Goslinski, T.; Sobotta, L. Titanium dioxide nanoparticles: Prospects and applications in medicine. *Nanomaterials* **2020**, *10*, 387. [CrossRef]
- 55. Rochkind, M.; Pasternak, S.; Paz, Y. Using dyes for evaluating photocatalytic properties: A critical review. *Molecules* **2014**, *20*, 88–110. [CrossRef]
- Feng, X.; Zhang, S.; Wu, H.; Lou, X. A novel folic acid-conjugated TiO₂-SiO₂ photosensitizer for cancer targeting in photodynamic therapy. *Colloids Surf. B Biointerfaces* 2015, 125, 197–205. [CrossRef] [PubMed]
- 57. Zaleska, A. Doped-TiO₂: A review. Recent Pat. Eng. 2008, 2, 157–164. [CrossRef]
- 58. Aristidis, T.; Demetrious, P.; Taxiarchis, K.N.; Anca, O.D.; Daniela, C.; Marco, V.; Marina, G.; Ronald, N.K.; Charalampos, M.; Michael, A.; et al. COVID-19, an opportunity to reevaluate the correlation between long-term effects of anthropogenic pollutants on viral epidemic/pandemic events and prevalence. *Food Chem. Toxicol.* **2020**, *141*, 111418.