Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

Article
Volume 12, Issue 2, 2022, 2548 - 2559
https://doi.org/10.33263/BRIAC122.25482559

Protective Effects of Nanosof® Suspension on Cultured Cells Exposed to H_2O_2

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Received: 23.04.2021; Revised: 28.05.2021; Accepted: 3.06.2021; Published: 24.06.2021

Abstract: Nanoparticle and nanomaterial-based treatments have improved a lot recently in terms of bioavailability, effectiveness, and reduced toxic and side effects. Many studies found a protective effect of fullerene C_{60} derivatives as potent free radical scavengers in biological systems and also showed neuroprotective properties when tested on *in vivo* models of ischemic stroke. This study assessed the antioxidant effects of Nanosof® powder suspension, an oxygenated fullerene compound, on various cell types exposed to exogenous free oxygen radicals. Cor.4U® cardiomyocytes and bEnd.3, BV-2, HEK293/hERG1 cell lines were treated with Nanosof® powder suspension alone or during exposure to 100 µM H_2O_2 for 24 h, in order to check the nanoparticle capacity to neutralize reactive oxygen species, using MTS or MTT to assess viability. We found no significant change in the viability of cells treated with Nanosof® compared to control. In the presence of H_2O_2, Nanosof® increased cell viability compared to H_2O_2 exposure alone. Nanosof® treatment showed no side effect; moreover, it exerted a protective effect on all three tested cell lines and Cor.4U® cardiomyocytes, indicating that treatment with this oxygenated fullerene may benefit various conditions.

Keywords: cardiomyocyte; endothelial cells; hydrogen peroxide; microglia; oxygenated fullerenes; viability assay.

1. Introduction

The field of nanotechnology evolved steadily during the last decade, providing a variety of alternative approaches or complementary medications [1-5]. New nanotechnology achievements [6-9] have contributed to a better understanding of the complex etiopathogenesis of some widespread diseases, particularly in neurology [10-18]. Several metallic and organic nanomaterials have been approved by the US FDA (United States Food and Drug Administration) at different clinical trial stages as diagnostic tools [19].

Biocompatibilization by conjugation of fullerene-based molecules has become a primary strategy in overcoming the intrinsic obstacles encountered by fullerenes to utilize them for biological purposes. Consequently, assays aiming to apply fullerenes in different physicochemical and pharmacological research areas extend at an alert pace, and breakthroughs and developments frequently occur [20-24].

1.1. Neuroprotective effects of fullerene C_{60} derivatives.

In order to improve the bioavailability and reduce the toxic and side effects, nanotechnology treatments have improved a lot in recent years. Neurodegenerative diseases are common among older people and a major social and medical problem. Receptors of blood-brain barrier (BBB) endothelial cells have been used as therapeutic targets for this kind of disease [25]. However, even though circulating fullerenes are in direct contact with vascular endothelial cells, few studies have addressed their effect on cerebral endothelial cells [26]. In particular, the capacity of fullerenes to modulate the BBB properties of brain-derived microvascular endothelial cells has not been investigated so far. Water-soluble polyhydroxylated fullerene derivatives known as fullerenols or fullerols might counteract oxidative stress via the extracellular-signal-regulated kinase (ERK1/2) and nuclear factor-κB (NFκB) pathways, and thus can protect microvascular endothelial cells under inflammatory conditions [27].

Due to unique physicochemical properties, relative biocompatibility, and versatility in surface modification with various molecules, fullerene C_{60} and its derivatives have been investigated as a novel way to improve modalities used to diagnose, monitor, and treat certain chronic diseases [28-30]. Due to their pharmacological properties, water-soluble fullerene derivatives are some of the compounds used to treat and prevent acute and chronic neurodegenerative disorders [31].

Previous studies have shown that activated microglia release neurotoxic factors that can cause toxicity to neurons. This indirect toxicity could also play important roles in developing neurodegenerative diseases, including prion diseases [32,33]. A study demonstrated that $C_{60}(OH)_n$ (usually n = 24) inhibits cellular prion protein PrP(106-126)-induced excessive production of pro-inflammatory cytokines and toxic molecules in BV-2 rat microglial cells [31]. $C_{60}(OH)_n$ does not exert cytotoxicity to BV-2 cells at a concentration less than 100 μM, and a similar concentration range was used to evaluate biological activities of $C_{60}(OH)_n$ *in vitro* [34-36]. In general, BBB restricts the entry of many potentially therapeutic agents into the brain. But there is evidence showing that water-soluble C_{60} derivatives delivered by intraperitoneal injection or oral administration can cross BBB *in vivo*, showing significant benefits in neurological disorders. $C_{60}(OH)_n$ are also effective in modulating the activation of microglial cells and reducing indirect toxicity *in vitro*. The increasing use of fullerene materials warrants toxicological investigations *in vivo*. $C_{60}(OH)_n$ administered by intraperitoneal injection or oral administration might reduce inflammation and confer neuroprotection *in vivo* in a model of prion disease. The anti-inflammatory activity of fullerenol $C_{60}(OH)_{24}$ was associated with activation of Nrf2/ARE (transcription factor NF-E2-related factor 2/antioxidant responsive element) pathway, providing insight into the underlying mechanism of neuroprotective effects of C_{60} derivatives. Although more studies are needed to further explore and confirm initial findings, these results might help design and utilize fullerenes and their derivatives as therapeutic approaches for the treatment of prion disease and an attractive strategy to prevent neurodegenerative diseases associated with it inflammation [31].

Synthesis of water-soluble fullerene derivatives is thought to be a promising strategy to protect cells against apoptosis by attenuating oxidative stress [37]. A water-soluble C_{60}-GSH derivative was synthesized and its cell-protective effects were proved, including inhibition of intracellular reactive oxygen species (ROS) production, improved cell viability and remarkable alleviation of H_2O_2-induced cell apoptosis without evident toxicity in HEK293T cells [38].

1.2. Antiischemic effects of fullerene C_{60} derivatives.

Many studies found protective effects of fullerenol C_{60} derivatives as potent free radical scavengers in biological systems [39]. Some studies confirmed that pretreatment with Frl (one of these nanoparticles) completely prevented heart rate variability (HRV) alterations induced by doxorubicin (an anti-cancer drug known to exert cardiotoxic side effects), decreased oxidative stress and normalized myocardial histopathological score, therefore it may play a role in the prevention of acute doxorubicin-induced cardiotoxicity [40-42]. Another remarkable effect is activation by C_{60} of mitogen-activated protein kinase (MAPK) expression level resulting in improved brown adipose stem cells survival, proliferation and cardiomyogenic differentiation [43].

A single C_{60} sphere can catch as many as 34 methyl radicals, leading to a description of this molecule as a „radical sponge" [44]. Free radical-scavenging properties have been confirmed by quantum-mechanical simulations [45]. Fullerene derivatives also cause the recovery of glutathione (GSH) [46] and nitric oxide (NO) [47], thus supporting free radicals removal. In addition, fullerene derivatives have shown neuroprotective properties *in vivo* models of ischemic stroke [48].

1.3. Characterization of Nanosof®.

Nanosof® is an oxygenated fullerene chemically heterogeneous product (over 80% elemental oxygen content) obtained by original methods from carbon products of animal origin. The product was previously tested in multiple *in vitro*, *in vivo*, and clinical trials and was shown to exert several remarkable therapeutic effects, most notably an increased scavenging effect on free oxygen radicals and potentiating effects on the antioxidant properties of other antioxidants of natural origin. These properties are exploited in the commercially available product Nanoxyn alpha, a product approved by FDA as a food supplement and delivered on the US market (more data available on the company website https://nanoxyn.ro/en/).

1.4. Oxidative stress.

Oxidative stress, which can be induced by endogenous or exogenous free radicals such as H_2O_2, plays a crucial role in many acute and chronic diseases, including ischemia-reperfusion injury [49], viral infections [50], neurodegenerative diseases [51-53] and cancer [54], therefore a large variety of natural or artificial antioxidants have been tested as therapeutic agents [55-57]. Studies aiming to resolve this problem have been continued for decades, but the underlying mechanisms are still not fully understood. H_2O_2, a significant ROS resource, has been widely used to induce exogenous oxidative stress in many different cell models. Previous studies indicated that H_2O_2-induced oxidative stress and cell apoptosis were associated with ROS generation and intracellular calcium mobilization regulated by the Bcl-2 family and subsequent caspase cascade activation [58,59]. Indeed, free radicals scavengers and antioxidants have been used to attenuate H_2O_2-induced cell apoptosis, which has attracted considerable attention recently [38].

1.5. Objectives.

This study aims to assess the antioxidant effects of Nanosof® powder suspension, an oxygenated fullerene compound, on various cell types exposed to exogenous free oxygen radicals in order to certify new efficient treatments in various conditions, following *in vitro*

experiments. Different human or animal cell lines, including bEnd.3 (mouse endothelial cells), BV-2 (rat microglia) and HEK293T/hERG1 (human embryonic diploid), and Cor.4U® cardiomyocytes (human stem cell-derived), were treated with Nanosof® powder suspension alone or in the presence of hydrogen peroxide (H_2O_2) in order to check the nanoparticle scavenging capacity using MTS or MTT viability assays.

2. Materials and Methods

2.1. Study design.

This study was performed entirely *in vitro* on primary cultures or cell lines and did not include experiments on humans or animals. All experiments were performed in triplicate. The investigations were carried out following the rules of the Declaration of Helsinki of 1975 (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2013, and were approved by the Ethics of Scientific Research Commission of the University of Bucharest (approval 115/11.11.2016).

After seeding the cells in 12 wells of a 96-well plate for each cell type in a total volume of 100µl/well, the plate was left overnight in the incubator at 37 °C. 3 wells were used for background read-outs and were filled with 100µl of medium each, devoid of cells.

		H_2O_2 (-)			H_2O_2 (100 µM)								
		1	2	3	4	5	6	7	8	9	10	11	12
Nanosof (-)	A												
Nanosof (+)	B												
Nanosof (-)	C	Bg	Bg	Bg									
	D												
	E												
	F												
	G												
	H												

Figure 1. Experimental cell culture pattern in 96-well plates for the MTS or MTT cell viability test.

During the following day, the medium of the 12 cell-seeded wells for each plate was discarded and replaced with medium supplemented with specific factors, according to the pattern specified in Figure 1. Thus, in each plate, 3 of the 12 cell-containing wells were treated for 24 hours with normal cell culture medium (no treatment added, or double-negative control); 3 wells were treated for 24 hours with 100 µM H_2O_2; 3 wells were treated for 24 hours with 100µM H_2O_2 with Nanosof® suspension; 3 wells were treated for 24 hours with Nanosof® suspension only; for the 3 wells used for background spectrophotometer read-outs, the medium was also replaced with fresh normal medium. Hydrogen peroxide (H_2O_2) treatment is a well-known and widely used method of exposure of biological systems to exogenous free oxygen radicals. Therefore cells exposed to 100 µM H_2O_2 (for 24 h) were used as a positive control, while those exposed only to Nanosof® suspension were used as a control for effects on cell viability of this compound *per se*.

After 24 hours of incubation in specific conditions at 37 °C, viability assays were performed.

2.2. Experimental procedures.

2.2.1. Preparation and cultivation of cells.

Cells from the Balb/c mouse cell line bEnd.3 (passage 10-20; #ATCCr CRL-2299tm, American Type Culture Collection, VA, USA) were maintained in DMEM (Gibco, 12491-015) supplemented with 10% fetal bovine serum (Gibco, 10270-098), 1% Penicillin/Streptomycin (Gibco 15140-122), 2 mM L-Glutamine (Gibco, 25030-081), and 250 µg/ml Amphotericin B (Gibco, 15290-026) in a 5% CO_2 atmosphere at 37°C, as described previously [60].

BV-2, a retrovirus-immortalized microglia cell line primarily obtained from the rat brain, was maintained in DMEM (10-013-CVR, Corning) + 10% fetal bovine serum (F7524, Gibco) + 1% Penicillin/Streptomycin (P4458, Sigma) in a 5% CO_2 atmosphere at 37°C.

HEK293T/hERG1 (human embryo kidney 293 cells stably transfected with the *human ether-à-go-go-related gene 1* K^+ channel), a human diploid cell line extracted from the embryonic kidney (metanephros), expressing the main transmembrane subunit of the human cardiac ion channel hERG1 in combination with a neomycin/geneticin (G-418) resistance element, was maintained in DMEM (Sigma, D5523) supplemented with 1% Glutamax (Gibco, 35050), 0.5% Penicillin-Streptomycin (Sigma, P4458), 7.4 mM D(+)-Glucose (Merck, 1.08337.0250), 10% fetal bovine serum (Gibco, 10270-098), and 300 µg/ml G-418 (Sigma, A1720) in a humidified incubator at 5% CO_2 and 37°C.

Cor.4U® human stem cell-derived cardiomyocytes (Axiogenesis AG, Cologne, Germany, currently part of Ncardia) were thawed and cultured at high density on Geltrex®-precoated wells of 24-well plates in Cor.4U® medium according to manufacturer's instructions, in a 5% CO_2 atmosphere at 37 °C.

bEnd.3, BV-2 and HEK293T/hERG1 cell lines were detached from 25 cm^2 angled cell culture flasks (Nunclon® Surface) following Trypsin-EDTA treatment (3 min at 37 °C) with sterile tips, centrifuged briefly (5 min at 1000 g), resuspended in control cell culture medium, and cell density was counted with a Bürker-Türk chamber and adjusted such as to plate 5000 cells per well in a 96-well cell culture plate with a flat bottom, up to a 100-µl final volume for each well. Each cell line was disposed of in 12 wells following a predefined pattern (Figure 1). The cells were grown overnight in a humidified incubator (37 °C, 5% CO_2). The same procedure was applied for Cor.4U® cardiomyocytes according to manufacturer's instructions.

2.2.2. Solutions and chemicals.

The Nanosof® powder was a kind gift from Nanotech Health SRL (Ilfov, Romania). Preparation of the Nanosof® powder suspension used for these experiments was performed according to manufacturer's instructions, mixing 1 mg of pure powder (with rusty red color and paramagnetic properties) per 1 ml of cell culture medium in a plastic tube, followed by moderate centrifuging (1 min at 3200 rpm into an Eppendorf microcentrifuge). The pellet was discarded, while the supernatant was collected with a sterile syringe and subsequently filtered through 0.22 µm filters (Millipore Millex® GP Filter units with Millipore Express® PES membranes). Before centrifuging, the Nanosof® primary suspension was vortexed.

2.2.3. Nanosof® administration.

The cells were cultured at a proper density of 5000 cells/well in 96-wells plates. The cultures were maintained for 24h at 5% CO_2 atmosphere at 37°C. The next day the cells were

treated with sterile-filtered Nanosof® suspension in culture medium following the manufacturer's instructions and/or with 100µM H_2O_2 for 24h. The following day the medium was removed and replaced with MTT or MTS, used for cell viability assay.

2.2.4. MTT assay for cell viability.

For assessment of the number of viable cells in treated and control groups, MTT (methylthiazolyldiphenyl-tetrazolium bromide) assay was performed after Nanosof® treatment. An MTT assay is a qualitative index of cell viability. Mitochondrial and cytosolic dehydrogenases of living cells reduce the yellow tetrazolium salt (MTT) to produce a purple formazan dye that can be detected spectrophotometrically [61].

The viability of the cells was determined by performing an MTT assay 24 h after treatment. The cells were seeded at a density of 5000 cells/well on 96-well plates in 100 µL of medium and incubated at 37 °C for 24 h. Afterward, the medium was replaced with sterile-filtered Nanosof® suspension in medium and/or hydrogen peroxide in the medium at 100µl/well total volume, and the cells were incubated overnight at 37 °C. The next day the medium was replaced with 100µl/well culturing medium (serum-free) and MTT (Sigma, M2128) reduction was initiated by adding MTT to a final concentration of 1 mg/ml per well. After 3-4 h of incubation at room temperature, the reaction was stopped by removing the MTT-containing medium and adding 100-150 µl of DMSO (Dimethyl Sulfoxide) to each well to dissolve the formazan crystals. The plate was shaken for 20 min and the amount of cellular MTT formazan product was determined by measuring absorbance using a FlexStation 3 microplate reader (Molecular Devices, San Jose, CA) at a test wavelength of 570 nm and a reference wavelength of 650 nm.

For normalization, the absorbance readings were divided by the adjusted averaged absorbance reading of untreated cells in control wells (double-negative control, not exposed to H_2O_2 or Nanosof® suspension) to obtain the proportion of cell viability. Background absorbance was corrected using triplicate sets of wells containing medium only (no cells) and MTT reagent as per the experimental well.

2.2.5. MTS assay for cell viability.

MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay represents an *in vitro* cell viability test based on an organic tetrazolium compound and a coupling reagent via electron transfer [phenazine ethosulfate; PES], featuring high stability, and thus being able to combine with MTS to form a stable solution. Spectrophotometric absorbance at 490 nm, measured at 1-4 hours following application in a well, is proportional to the number of viable cells. Cells were seeded at 5000 cells/well in 96-well plates and left to attach overnight at 37 °C. After 24 h the cells were treated with sterile-filtered Nanosof® suspension (1 mg/ml) and/or hydrogen peroxide (100 µM) overnight at 37 °C.

20 µl of MTS solution were added to each well, and the plate was left at room temperature, in sterile conditions. Absorbance at 490 nm (for optical density - OD) and at 650 nm (for background) was read with the plate spectrophotometer at 1 h, 2 h, and 4 h since MTS addition. After 3 hours, the content of each well was mixed by gentle aspiration and release with a laboratory pipette 5-fold.

Reduction in cell viability relative to untreated cells was measured 24 h after the exposure, by MTS assay, following the manufacturer's protocol. The absorbance of each well was measured with a FlexStation 3 microplate reader (Molecular Devices) at 490 nm and at 650 nm for background. The proportion of viable cells in each well was normalized by division to the average of control wells. Triplicate sets of wells without cells incubated with MTS solution were used for background subtraction. All treatments are summarized in Table 1.

2.2.6. Statistical analysis.

All data measured by the microplate reader (FlexStation 3, Molecular Devices) were transferred and processed in Microsoft Excel. Statistical analysis of data was performed using a *two-tailed unpaired Student's t-test* and applied to evidence statistically significant differences between groups. Graphs in Figure 2 represent statistical significance as moderate * ($p < 0.05$), high ** ($p < 0.01$), and very high *** ($p < 0.001$). For $p > 0.05$, the tests were considered non-significant (ns). All data are reported as means ± SEM and are normalized relative to averaged control values (H_2O_2(-)N(-)).

3. Results and Discussion

3.1. The effect of Nanosof® on bEnd.3 cells viability using MTS assay.

The MTS test showed that in the presence of H_2O_2, Nanosof® increased bEnd.3 cell viability (relative viability 1.215 ± 0.069, $n = 3$, $p < 0.01$) compared to H_2O_2 exposure alone (relative viability 0.554 ± 0.074, $n = 3$). Compared to control (1 ± 0.015, $n = 3$), the presence of Nanosof® alone increased cell viability (1.138 ± 0.025, $n = 3$, $p < 0.01$) (Figure **2**A).

3.2. The effect of Nanosof® on BV-2 cells viability using MTS assay.

In the presence of H_2O_2, the MTS test showed that Nanosof® powder suspension increased BV-2 cell viability (relative viability 0.87 ± 0.087, $n = 3$, $p < 0.05$) compared to H_2O_2 exposure alone (relative viability 0.507 ± 0.099, $n = 3$). Nanosof® treatment showed no side effect ($p > 0.05$ for H_2O_2(-)N(-) *vs.* H_2O_2(-)N(+)); moreover, it exerted a protective effect against oxidative stress ($p > 0.05$ for H_2O_2(+)N(+) *vs.* H_2O_2(-)N(-)) (Figure **2**B).

3.3. The effect of Nanosof® on HEK293T/hERG1 cells viability using MTS assay.

The MTS assay showed that in the presence of H_2O_2, Nanosof® powder suspension increased HEK293/hERG cell viability (relative viability 0.506 ± 0.094, $n = 3$, $p < 0.05$) compared to H_2O_2 exposure alone (relative viability 0.058 ± 0.051, $n = 3$). Nanosof® treatment showed no side effect ($p > 0.05$ for H_2O_2(-)N(-) *vs.* H_2O_2(-)N(+)). Compared to control (1 ± 0.062, $n = 3$), the presence of Nanosof® powder suspension alone maintained good cell viability (relative viability 0.875 ± 0.069, $n = 3$, $p > 0.05$) (Figure **2**C).

3.4. The effect of Nanosof® on Cor.4U® cardiomyocytes cells viability using MTT assay.

In the presence of H_2O_2, Nanosof® powder suspension increased cell viability (relative viability 0.789 ± 0.042, $n = 3$, $p < 0.05$) compared to H_2O_2 exposure alone (relative viability 0.491 ± 0.096, $n = 3$), using MTT viability assay. However, compared to control (1 ± 0.093, $n = 3$), the presence of Nanosof® alone did not affect cell viability (0.93 ± 0.04, $p > 0.05$ for H_2O_2(-)N(-) *vs.* H_2O_2(-)N(+)) (Figure **2**D).

Our results for all four cell culture preparations tested in this experiment indicate that Nanosof® suspension effectively neutralized the toxic effect on cell viability of hydrogen peroxide without being toxic for the cells *per se*. Figure **2** (A, B and C) illustrates MTS normalized cell viability values (mean of 3 wells for each tested condition). Figure **2**D illustrates MTT normalized cell viability values (mean of 3 wells for each tested condition) at 4 h, following the dissolution of the formazan salt in DMSO. All absolute absorbance values are listed in Table 1.

Table 1. Cell viability test: absolute absorbance measured by the microplate reader for the 4 cell preparations tested.

Cell type	H_2O_2(-)N(-)			H_2O_2(+)N(-)			H_2O_2(-)N(+)			H_2O_2(+)N(+)			Test used
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	
bEnd.3	0.65	0.0098	3	0.36	0.048	3	0.74	0.016	3	0.79	0.045	3	
BV-2	0.69	0.0098	3	0.35	0.068	3	0.71	0.015	3	0.6	0.06	3	**MTS**
HEK293	0.16	0.0099	3	0.0093	0.0081	3	0.14	0.011	3	0.081	0.015	3	
Cor.4U®	0.57	0.053	3	0.28	0.055	3	0.53	0.023	3	0.45	0.024	3	**MTT**

H_2O_2(+) - cells exposed to H_2O_2 100 µM for 24 h
H_2O_2(-) - cells non-exposed to H_2O_2
N(+) - cells exposed to Nanosof® powder suspension in medium
N(-) - cells non-exposed to Nanosof®

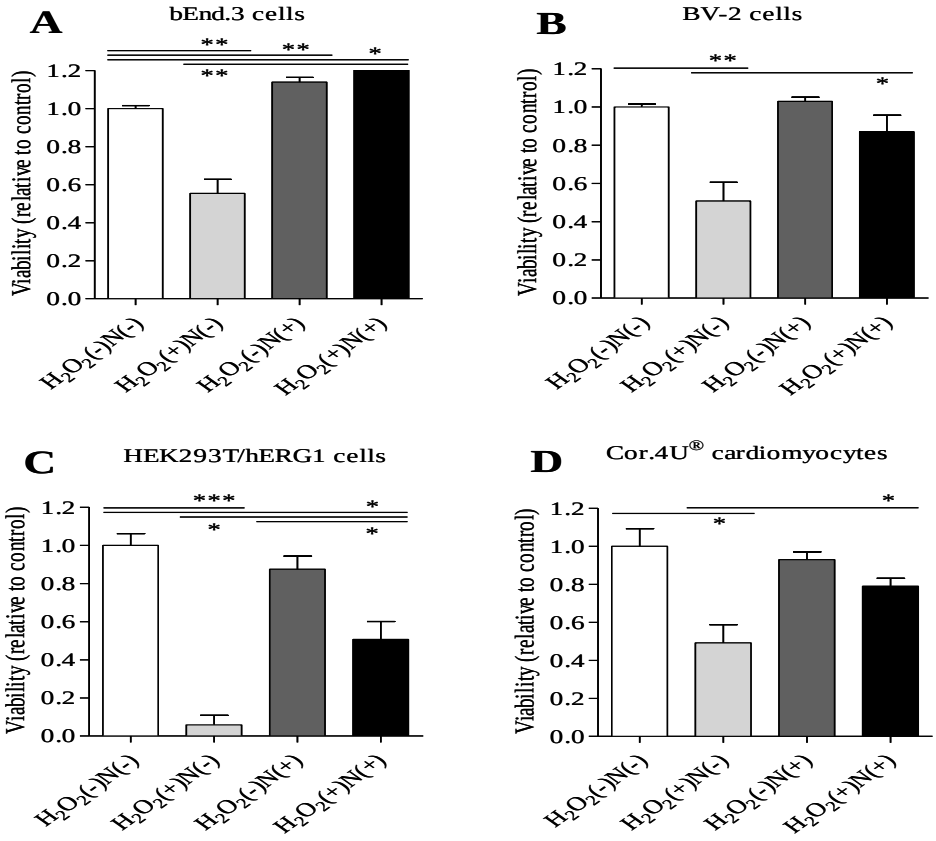

Figure 2. Effect of Nanosof® suspension on background cell viability and exposure to H_2O_2 for all four tested cell preparations. A. Effect of Nanosof® suspension on bEnd.3 cell viability; B. Effect of Nanosof® suspension on BV-2 cell viability; C. Effect of Nanosof® suspension on HEK293/hERG1 cell viability; D. Effect of Nanosof® suspension on Cor.4U® cardiomyocytes cell viability. For A, B and C, the values of absorbance at 490 nm following 4 h of exposure to MTS (mean for three wells/experimental condition) are shown; For D the values of absorbance at 570 nm following 4 h exposure to MTT, shortly after replacement with DMSO, are shown. Statistical significance is marked with * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. Error bars represent the standard error of the means (SEM).

Our research provides direct experimental proofs for the free oxygen radicals scavenging capacity of Nanosof® powder suspension, which remarkably ameliorates H_2O_2-

induced cell apoptosis without evident toxicity in all tested cell preparations, confirming previous results with fullerenes and fullerenols applied *in vitro* to hydrogen peroxide-exposed cells [34,36,38] or to *in vivo* ischemia-reperfusion models [27,46,48].

Our results showed no significant change in the viability of cells treated with Nanosof® powder suspension compared to control. In the presence of H_2O_2, Nanosof® increased cell viability compared to H_2O_2 exposure alone. Nanosof® treatment showed no side effect; moreover, it exerted a protective effect on all three tested cell lines and Cor.4U® cardiomyocytes, indicating that treatment with this oxygenated fullerene may be beneficial in various conditions.

Nanosof® suspension applied in the presence of H_2O_2 exerted beneficial effects, possibly via scavenging or otherwise neutralizing exogenous free oxygen radicals, in all four tested cell preparations. Nanosof® suspension was not toxic and did not significantly influence cell viability for all three tested cell lines.

The exogenous free radical exposure model chosen for this experiment was adequate, resulting in significant decreases in cell viability for all four tested cell systems.

4. Conclusions

Nanosof® suspension could be useful in cell culture experiments for enhancing cell viability and limiting the deleterious effects of free radicals. For example, it could be used in the cell culture medium of human-induced pluripotent stem cell-derived cardiomyocytes to limit the toxic effects of free oxygen radicals on various cellular components, for example, on L-type calcium channels. We will seek to perform further tests concerning the effects of Nanosof® suspension on biological properties such as cell migration capacity. Nanosof® may be a new antioxidant treatment with broad clinical applicability.

Funding

This study was funded from Competitiveness Operational Programme 2014–2020 project P_37_675 (contract no. 146/2016), Priority Axis 1, Action 1.1.4, co-financed by the European Funds for Regional Development and Romanian Government funds.

Acknowledgments

The authors gratefully acknowledge Cornelia Dragomir and Geanina Haralambie for their support.

Conflicts of Interest

The authors have no actual or potential conflict of interest associated with this work.

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