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ARAGONITE-NANOPARTICLES INDUCE BREAST CANCER CELL LINE THROUGH DOSE INCREASE REACTIVE OXYGEN SPECIES

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Abstract

This research was carried out to examine the mode of interaction of Doxorubicin Loaded Aragonite Calcium Carbonate Nanoparticles with the breast cancer cell, to understand the effect of these agents on cell organic molecules. Cockle shells, antibiotics combination, Phosphate buffered saline, Doxorubicin hydrochloride, intracellular Reactive Oxygen Species Assay kit Superoxide dismutase, were all used in the study. The results of the investigation indicate that treatment of MCF-7 cells with DOX-Ar-CC-NPs and DOX exhibited a dose-dependent effect on cell viability. The ability of DOX-NPs to induce apoptosis in MCF-7 cells was conflicting with the high potency of Aragonite Calcium Carbonate Nanoparticles in drug delivery.

KEYWORDS:
Oxidative stress; Doxorubicin; Nanoparticle; aragonite-calcium carbonate; Breast cancer; Cockleshell.

INTRODUCTION

Decades of research in science and engineering have led to the development of precise molecular structures and gadgets in the nanometre scales, and this usually is popularly referred to as nanotechnology [1]. The nanomaterial at a Nanoscale demonstrates new and distinctive properties which have facilitated its applications in medicine

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and life sciences [2]. This advancement has made Nanomaterials pretty for utilization for different items which has resulted in the development in the field of nanotechnology, because of the advantage guaranteed by the synthesis of nanomaterials into products [3]. Through the advancement of nanotechnology, the size impacts of particles have steadily been thought to be imperative [2]. Consequently, an enhanced potential threat, including exposure and risk evaluations, related to the introduction of nanomaterial is crucial to check its safety or toxicity [4]. Indeed, even though nanomaterials are as of now being generally utilized as a part of present-day innovation, there is a genuine absence of data concerning the human health and ecological effects of prepared nanomaterials [5]. The primary aim of the present work was to study how Ar-CC-NPs interact with tumor MCF-7 cell lines to understand the effect of such nanomaterial on organic cell molecules. In this study, we designed a nano anticancer-oxidative stress markers formulation using Doxorubicin-loaded Cockle-shell Aragonite nanoparticles DOX-Ar-CC-NPs, a hydrophilic anticancer drug. Our primary goal was to evaluate the oxidative stress markers by MCF-7 cell line following treatment. The designed Ar-CC-NPS-anticancer formulation was further evaluated for therapeutic efficacy against MCF-7 (Breast cancer) cells in vitro.

Materials and Methods

Synthesis, physicochemical characterization, and biocompatibility of Ar-CC-NPs and DOX-Ar-CC-NPs on MCF-7 Breast cancer cell-line Details on these aspects have been earlier published [13].

Cell cycle assay

Reactive oxygen species (ROS) assay

Intracellular reactive oxygen species (ROS) generation after the treatment of aragonite calcium carbonate nanoparticles (Ar-CC-NPS) was evaluated using 2,7–dichlorofluorescein diacetate (DCFH-DA) as described by the protocol and by Abdelaziz *et al.* (2018), Ahamed *et al.* (2016), and Rajabnia *et al.* (2018) with slight modification. Two techniques measured the ROS level; fluorometric quantitative assay and cell imaging. In preference to

fluorometric quantitative assay, 1 x 10 ^{5} cells/well were seeded in black cell culture fluorometric plates and allowed to attach on the surface for 24 hrs in a CO_2 incubator at 37°C. Furthermore, after cells were treated with different concentrations (5, 25, and 100 g/mL) of DOX, DOX-Ar-CC-NPs, Ar-CC-NPs, and Control for 24 hrs. After the exposure was completed, cells were washed with the media to each well twice-trice with DPBS before being incubated in 1 mL of working solutions of DCFH-DA for 30 min at 37°C. Thereafter, cells were lysed in an alkaline solution and centrifuged at 1500 g for 10 min. A 100 µL supernatant was transferred to a new suitable for fluorescence measurement at 480 nm excitation and 530 nm emissions utilizing the microplate reader (Synergy-HT, BioTek, USA). The values were presented as a percent of fluorescence intensity relative to control. A parallel set of cells (1 x 10^5 cells/well) in a 96-well plate suitable for fluorescence were analyzed for intracellular fluorescence using a fluorescence microscope (OLYMPUS CKX 41), with images taken at 200 x magnification.

Superoxide dismutase (SOD)

Cell cycle assay was evaluated using cycle-test Plus DNA reagent kit (BD Bioscience, USA). In brief, MCF-7 cells were seeded at 5 x 10^5 cells per well in six-well plates and incubated for 24 hrs, with equivalent concentration DOX alone for 24, 48, and 72 hrs. The cells were then treated with the IC_{50} of DOX, DOX-Ar-CC-NPs, Ar-CC-NPs (0.5 µg/mL), and control. The complete growth medium (Untreated Cells) served as the control. After each treatment, the adherent and floating cells were collected, and the seeded cells were suspended with 250 µL of trypsin buffer. After 5 minutes, 200 µL of trypsin inhibitor with 50 µL RNase buffer was added. Finally, the sample's cell was stained with 500 µL Propidium Iodide (PI) solution and incubated on ice for 30 minutes. The sample was run on a flow cytometer (Becton Dickinson, USA).

Statistical analysis

All statistical analysis was done using SPSS software (IBM SPSS Statistics version 23, USA) Comparisons between groups were determined using one-way analysis of variance (ANOVA), followed by post hoc group comparison

of Dennett's multiple range test, significance was attributed at $p<0.05$ unless indicated otherwise. All experiments were conducted at least three times.

Results

Reactive oxygen species assay

Quantitative data suggested that DOX-Ar-CC-NPs induced ROS generation in a dose-dependent manner ($p<0.05$) (Figure 1-2). Similarly, fluorescent microscopy data also showed that DOX-Ar-CC-NPs treated cells and DOX alone express a high intensity of green fluoresce DCF dye as compared to the Control and Ar-CC-NPs (Figure 1-2). Despite this, an increase in fluorescence intensity at higher concentrations is an indicator that DOX may generate more ROS as the concentration and time increases, which in due course results in cell damage.

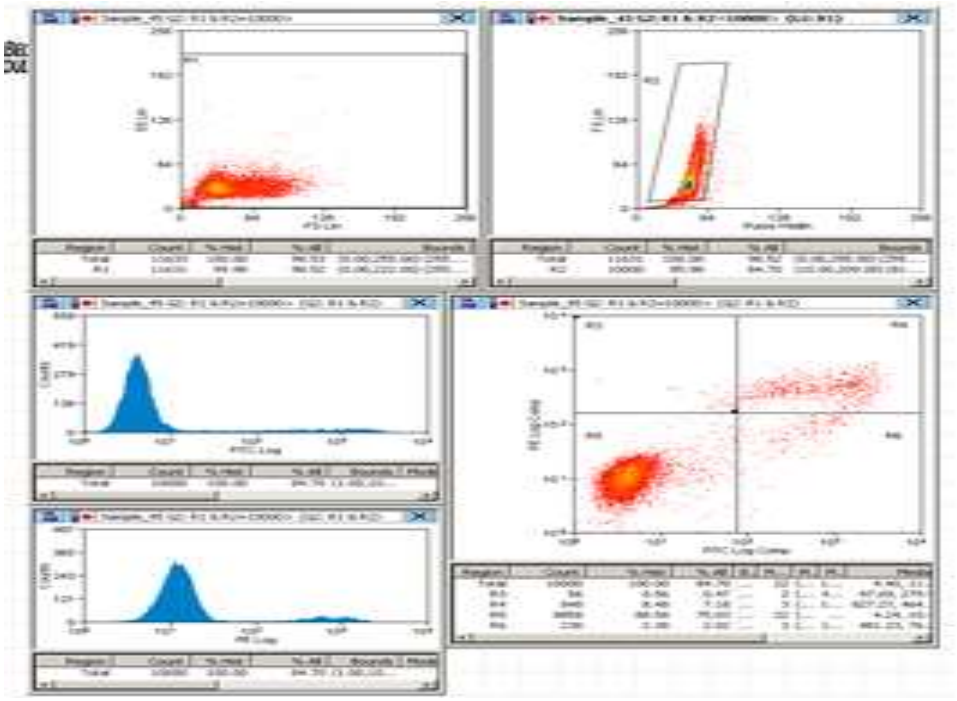

Figure 1: DOX-Ar-CC-NPs induced oxidative stress on MCF-7 cells exposed to DOX-Ar-CC-NPs, DOX alone, and Ar-CC-NPs at IC_{50} dosages 0.5 mg/ml for 72 hrs. Show A) Fluorescence microscopy images of ROS generation, B) Shows Hydrogen peroxide level.

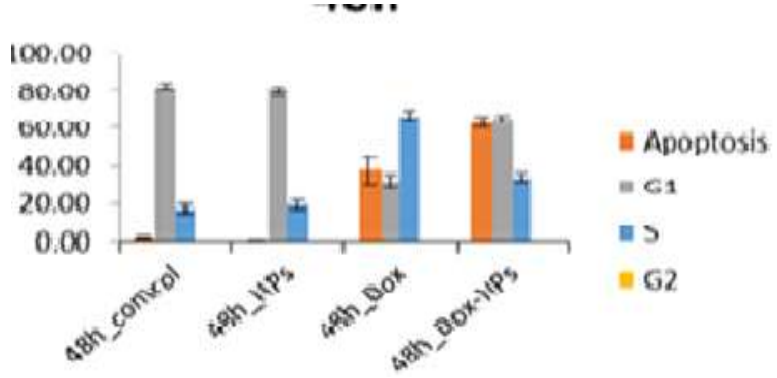

Figure 2: Cell cycle analyses of MCF-7 cells for 48 hrs in different groups. The flow cytometer histograms: (A) Control group, (B) Ar-CC-NPS treatment group, (C) DOX treatment group, (D) DOX-Ar-CC-NPS treatment group, (E) the quantification of cell cycle phase distributions of Mthe CF-the 7 cells in different groups. Note: DOX-Ar-CC-NPS treatment group killed more cells (refer to the percentage of apoptosis) as compared to DOX alone, with a similar trend at 48 hrs (p>0.05) compared with the control group.

Discussion

Further to the earlier detailed documentation on the synthesis, physicochemical characterization, and biocompatibility of Ar-CC-NPs and DOX-Ar-CC-NPs [13], this present study explored the ability of DOX and DOX-Ar-CC-NPs to induce clothe the toxicity by altering the intracellular oxidative condition in ROS production. The generation of ROS by DOX is generally considered a significant contributor to DOX toxicity and their formation, by exceeding the cellular defensive capability and causing oxidative damage to biomolecules [17,18]. Additionally, the overall ROS assay outcomes of the Ar-CC-NPs on MCF-7 provide use under stain oxygen metabolism where ROS sify to radicals or chemicals and molecules that comprise ROS compounds such as peroxides resulting from normal oxygen metabolic rate with vital roles in cell and homeostasis signaling [10,11]. This study was intended to normalize population units of the cecellines in which the results in this study (Figure.5.5) revealed that the DOX-Ar-CC-NPs treated MCF-7 cells exhibited higher reactive oxygen species as compared to the DOX alone treated cells. Reactive oxygen species generation of the DOX was significantly lower than on DOX-Ar-CC-NPs and demonstrated consistent ROS generation also in agreement with previous biocompatibility results further highlighting the remarkability of DOX-Ar-CC-NPs. For that reason, these results could be elucidated by the fact that the internalization of Ar-CC-NPs possibly induced oxidative stress which in turn triggered cell death demonstrated in the results. This study support evidence that induction of environmental stress drastically increases reactive oxygen species

levels triggering substantial cellular damage also identified as oxidative stress [11,12].

Conclusion

Treatment of MCF-7 cells with DOX alone and DOX-Ar-CC-NPs exhibited a dose-dependent validity on cell viability. The DOX-Ar-CC-NPs had a significant inhibitory effect on cell viability compared to DOX alone ($p<0.05$). A similar trend was noticed in oxidative stress markers. Besides, the results clearly showed that DOX-Ar-CC-NPs induced ROS generation of MCF-7 cells.

References

Ahamed, M.; Akhtar, M.J.; Alhadlaq, H.A.; Khan, M.A.M.; Alrokayan, S.A. Comparative cytotoxic response of nickel ferrite nanoparticles in human liver HepG2 and breast MFC-7 cancer cells. *Chemosphere* **2015**, *135*, 278–288, doi:10.1016/j.chemosphere.2015.03.079.

Dudefoi, W.; Villares, A.; Peyron, S.; Moreau, C.; Ropers, M.; Gontard, N.; Cathala, B. Nanoscience and nanotechnologies for biobased materials, packaging and food applications : New opportunities and concerns. *Innov. Food Sci. Emerg. Technol.* **2018**, *46*, 107–121, doi:10.1016/j.ifset.2017.09.007.

Colombo, S.; Beck-broichsitter, M.; Peter, J.; Malmsten, M.; Rantanen, J.; Bohr, A. Transforming nanomedicine manufacturing toward Quality by Design and microfluidics☆. *Adv. Drug Deliv. Rev.* **2018**, *128*, 115–131, doi:10.1016/j.addr.2018.04.004.

Liu, M.; Zheng, S.; Zhang, X.; Guo, H. Cerenkov luminescence imaging on evaluation of early response to chemotherapy of drug-resistant gastric cancer. *Nanomedicine Nanotechnology, Biol. Med.* **2018**, *14*, 205–213, doi:10.1016/j.nano.2017.10.001.

Elena, A.; Vidal, V.; Carrière, M.; Hiram, A.; Levard, C.; Chaurand, P.; Sarret, G.; Elena, A.; Vidal, V.; Carrière, M.; et al. Silver nanoparticles and wheat roots : a complex interplay To cite this version : HAL Id : hal-01516961. **2018**.

Hamidu, A.; Moorish, A.; Mansor, R.; Shameha Abdul Razak, I.; Danmaigoro, A.; Zubair Jaji, A.; Abu Bakar, Z. Modified methods onanoparticlees synthesis in pH-sensitive nano-carriers production for doxorubicin delivery on MCF-7 breast cancer cell line. **2019**, doi:10.2147/IJN.S190830.

Zhang, Y.; Ma, P.; Wang, Y.; Du, J.; Zhou, Q.; Zhu, Z.; Yang, X.; Yuan, J. Biocompatibility of Porous Spherical Calcium Carbonate Microparticles on Hela Cells. *World J. Nano Sci. Eng.* **2012**, *02*, 25–31, doi:10.4236/wjnse.2012.21005.

Saleha, A.A.; Khor, K.H.; ZZunigaZ.; Jalila, A. Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia. **2015**, *27*, 7–11.

Seabra, A.B.; Durán, N. Nanotoxicology of Metal Oxide Nanoparticles. **2015**, 934–975, doi:10.3390/met5020934.

Zhang, Y.; Gu, A.Z.; Xie, S.; Li, X.; Cen, T.; Li, D.; Chen, J. Nano-metal oxides induce antimicrobial resistance via radical-mediated mutagenesis. *Environ. Int.* **2018**, *121*, 1162–1171, doi:10.1016/j.envint.2018.10.030.

Anwar, A.; Gould, E.; Tinson, R.; Iqbal, J.; Hamilton, C. Redox Modulation at Work : Natural Phytoprotective Polysulfanes From Alliums Based on Redox-Active Sulfur. **2018**, 397–407.