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Cytotoxic effects of CeO_2 NPs and β-Carotene and their ability to induce apoptosis in human breast normal and cancer cell lines

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Abstract

Cerium Oxide Nanoparticles (CeO_2 NPs) and β-carotene are natural-source products that have recently gained an increased interest as pharmaceutical additives because of their effectiveness in living systems, but the behavior of these substances varies according to factors and conditions. The above mentioned materials were evaluated in breast normal (HBL-100) and cancer cell lines (CAL-51 and MCF-7) by different techniques ; MTT assay for studying cytotoxic effects, morphological changes, sqPCR, including gene expression of caspases 8 and 9, and P53. All experiments were conducted on cell lines by the use of the materials alone as well as their combination.

The results of the MTT assay showed that the two materials (CeO_2 NPs and β-carotene) had a toxic effect on the cell lines, and the toxicity of the materials was concentration-dependent. The half maximal inhibitory concentration (IC_{50}) values of CeO_2 NPs and β-Carotene, as observed by the effects in all cell lines, have significant differences.

Our findings showed that CeO_2 NPs has a higher toxicity on the HBL-100 and MCF-7 cell lines compared with β-Carotene. The toxic effects of each material alone and combined together were reflected on cell morphology by the appearance of irreversible cytopathic changes in the treated cells. The two materials had the ability to cause cellular death and DNA damage, inducing the external pathway of apoptosis. A significant increase in the gene expression of caspase 8 appeared at $P \leq 0.05$. Collectively, the CeO_2 NPs and β-Carotene have an inhibitory effect on both breast cancer and normal cell lines. We conclude the both materials have genotoxic effects on normal and cancer cell lines.

Keyword: CeO_2 NPs, β-Carotene, cell lines, cytotoxic effect

التأثيرات السمية لـ CeO_2 NPs و β−Carotene وقدرتها على حث الموت الخلوي المبرمج في الخطوط الخلوية الطبيعية والسرطانية للثدي البشري

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الخلاصة

تعد مادتي الـ CeO_2 والـβ−Carotene من المنتجات ذات المصدر الطبيعي التي تزايد الاهتمام بها مؤخرا كمكملات صيدلانية لفعاليتها في الأنظمة الحية , لكن سلوك تلك المواد يختلف تبعاً للعوامل والظروف.

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تم تقييم فعالية المواد سابقة الذكر في خطوط الخلايا الطبيعية (HBL-100) و السرطانية (CAL-51 و MCF-7) للثدي البشري بواسطة تقنيات مختلفة ؛ تقنية MTT assay لدراسة السمية الخلوية , دراسة التغيرات المظهرية للخلايا, دراسة التعبير الجيني لجينات caspase 8 و caspase 9 و p53 بواسطة تقنية sqPCR . أظهرت نتائج الـMTT assay إن مادتي الـCeO_2 NPs والـβ-Carotene لهما تأثير سام على الخطوط الخلوية , وتزداد سميتهما بزيادة التركيز. وقد اظهرت التراكيز المثبطة لنصف الخلايا IC_{50} من مادة CeO_2 NPs ومادة β-Carotene فرقاً معنوياً بين الخطوط الخلوية. وأظهرت النتائج إن الـ CeO_2 NPs أعلى سمية على خطوط خلايا HBL-100 و MCF-7 من الـβ-Carotene . انعكس التأثير السمي للمادتين على مظهر الخلايا المعاملة بهما من خلال ظهور تغيرات مظهرية مرضية غير انعكاسية على الخلايا. وان لكلتا المادتين القدرة على إحداث الموت الخلوي من خلال تضرر الـDNA وحث المسار الخارجي للموت الخلوي المبرمج, إذ ظهرت زيادة معنوية في تعبير جين caspase8 عند مستوى معنوية $P\leq0.05$. استنتجت الدراسة ان لمادتي CeO_2 NPs وβ-Carotene لهما تأثير مثبط على الخطوط الطبيعية والسرطانية للثدي البشري. كما استنتجت الدراسة ان لكلتا المادتين سمية جينية على الخطوط الطبيعية و السرطانية.

Introduction

Nature is the main source of bio-effective materials that are used for the treatment of different diseases, such as cancers [1].

Nano particles are natural materials which have a potential effect against tumor [2]. It is considered as an important candidate for the treatment of some oxidative diseases, such as neurodegenerative disease, ischemic cardiopathy, ocular disease, diabetes, and cancer [3]. Studies reported that CeO_2 NPs have various levels of cytotoxicity. Some studies indicated that CeO_2 NPs have low toxicity [4], while others suggested that CeO_2 NPs cause high toxicity that can lead to cellular death [5]. Other researches pointed to the role of CeO_2NPs in inhibiting certain types of cancer cell lines [6]. Despite these results, there is a paucity of information on the role of CeO_2NPs against different type of tumors.

Over recent years, phytochamicals were used as chemotherapy for different diseases [7]. The redox role of β-carotene was proven by previous studies [8]. Also, few previous investigations revealed that β-carotene has the potential as an antitumor compound [9]. Some work showed that the higher the concentration of β-carotene, the higher the rate of inhibition of cancer cells [7, 10].

The present study aimed to detect the roles of CeO_2 NPs and β-Carotene in breast cancer and normal cell lines, i.e. whether they have cytotoxic effects on cell lines or not. In addition, we aimed to investigate the combined effects of CeO_2 NPs and β-Carotene on the cell lines. The present study also aimed to investigate the possible ability of the two materials to induce apoptosis in breast cell lines.

Materials and methods

Cell maintaining

The cell lines, HBL-100 normal human breast epithelial cell line and tow human breast cancer cell lines that included CAL-51 and MCF-7, were obtained from the tissue culture laboratory in the College of Education for Pure Sciences of Basra University. The cells were maintained in RPMI 1640 culture medium with 10 % fetal bovine serum (FBS, Euroclone – Italy) and 100 mg/ml streptomycin/ampicilin at 37 °C and 5 % CO_2. After trypsinization, the cells were resuspended with RPMI with 10% FBS in a new T-25 flask , then incubated at 37 °C and 5 % CO_2 .

Cytotoxicity assay

The MTT assay was used to study viability of cell lines [11]. Briefly, the cells, after resuspension, were seeded in 96 well plates at $1x10^4$ with 100 ul RPMI medium supplemented with 10 % fbs, then incubated at 37 °C with 5 % CO_2 in a humidified incubator for 24 h. The series dilutions of CeO_2 NPs (<5nm) were prepared by serum free medium ,

while β-Carotene was prepared by serum free medium with less than 1% DMSO (Dimethyl sulfate). Volumes of 100 µl of CeO_2 NPs (<5nm) (0, 2.5, 10, 20, 30, 50, 90 µg/ml) and β-Carotene (0, 5, 15, 50, 150, 300, 500 µg/ml) were then added. Triplicates of each concentration of the treated and untreated cells were used, then the plates were incubated at 37° C with 5 % CO_2 in a humidified incubator for 72 h. The cell viability was calculated as follows: t/c×100, where t is treatment and c is control. Experiments were repeated three times. Then, the half maximal inhibitory concentration (IC_{50}) was determined using Graphpad Prism 8.0.1 software [12].

Combination effect

To study the combined effect of β-C and CeO_2 NPs, the MTT assay was also utilized [11]. Then, the cells were treated with a combination of CeO_2 NPs + β-C with IC_{50} values of 20+50 µg/ml and 50+300 µg/ml of CeO_2 NPs + IC_{50} β-C. The plates were incubated at 37 °C with 5 % CO_2 in a humidified incubator for 72 h. The cells inhibition was calculated as in the formula: *(c-t/c)×100*, where *t* is treatment and *c* is control [12] .

Then, the data were analyzed by the Compusyan Isobologram sofetware, Chou-Talalay method [13].

Morphologic study

The cells were seeded at $5x10^4$ cells/well in a slide chamber, incubate for 24 h, then treated with the IC_{50} of CeO_2 NPs and β-C for 72 h. Then, the cells were stained with Haematoxylin & Eosin (H&E) for morphological changes study. Briefly, the old medium was first discharged. After the slides were passed through a serial concentration (70, 90, and 100 %) of ethanol, they were treated with Haematoxylin and Eosin stains, respectively, for one minute each, covered with a cover slide, and examined by a light microscope [14]. The Acridin Orange / Ethidium Bromide (AO/EB) stains were used for the cytopathic study. Briefly, the medium was discharged and 150 µl of AO/EB was added for 20 second, then the slide was covered with a cover slide and examined by a fluorescent microscope [15].

DNA fragmentation

DNA fragmentation was performed by agarose gel electrophoresis [16]. Polluted cells were lyses by using the GENAID DNA extraction kit (Genaid Biotech Ltd., Taiwan). The extracted DNA was loaded on 1.5 % agarose gel. The gel was powered at 85 volts for 55 min.

Gene expression analysis

The total RNA was extracted from untreated and treated cells by utilizing the TriRNA Pure Kit (GEBEzol^{TM} , USA). The cDNA was prepared by utilizing the BIONEER Accu Power® kit (BIONEER, USA) . The expression of caspases 8 and 9 and p53 genes was evaluated by sqPCR. Briefly, the total volume per reaction (12.5 µl qPCR master mix, 1 µl forward primer, 1 µl reverse primer, 5.5 µl nuclease-free water, and 5 µl cDNA) was amplified using a thermo cycler, then PCR products were elecrophoresed using 1.5 agarose gel. After visualization, bands were analyzed using an Image Lab Bio-Rad V6 software. The expression of each sample was studied by dividing target gene by housekeeping gene values . The sequences of the primers are shown in Table 1.

Table 1-The sequences of the sets of primers

Sequence	Target genes	Reference
5-GTTTGAGGACCTTCGACCAGCT-3 129bp	Hs_Caspase 9_FWD4	[17]
5-CAACGTACCAGGAGCCACTCTT-3	Hs_ Caspase 9_REV4	
5-CCTCAGCATCTTATCCGAGTGG-3 128bp	Hs_P53_FWD4	[17]
5-TGGATGGTGGTACAGTCAGAGC-3	Hs_P53_REV4	
5-CATCCAGTCACTTTGCCAGA-3 128bp	Hs_Caspase8_FWD	[18]
5-GCATCTGTTTCCCCATGTTT-3	Hs_Caspase8_REV	
5'-ATCTGTCAATCCTGTCCGTGT-3'	R Hmn rRNA 18s	[19]
5'-GGAGTATGGTTGCAAAGCTGA -3'128bp	F Hmn rRNA 18s	

Statistical Analysis

IC_{50} values for β-Carotene and CeO_2 NPs for each cell line were compared using SPSS software for T test. IC_{50} values for each material were compared between the cell lines using SPSS software for the One Way Analysis of Variance (ANOVA). The values of gene expression were analyzed using SPSS software for T test.

Results and Discussion

Cytotoxicity of CeO_2 NPs

The results of the current study showed that CeO_2 NPs (<5 nm) had effects on the viability of CAL 51, MCF-7, and HBL-100 cell lines. The viability of cells was decreased in a concentration-dependent manner, as shown in Figure 1 A. The values of IC_{50} were 46.21 µg/ml for CAL-51, 43.23 µg/ml for MCF-7, and 39.82 µg/ml for HBL-100 cell lines (Figure 1-B). The results collectively showed that CeO_2 NPs (<5 nm) had a similar trend of cytotoxic effect on all cell lines (malignant and normal). This cytotoxic effect may be attributed to the small size of CeO_2 NPs. Our finding corresponds with previous studies [20, 21, 22]. Some studies showed the the small size of CeO_2 NPs had the ability to generate Reactive Oxygen Species (ROS) [23, 24 , 25]. The mechanism of ROS generation is dependent on the chemistry of CeO_2 NPs (Ce^{+3} / Ce^{+4} valence ratio) [26 , 27]. ROS plays multiple functions in cellular biology. ROS generation is a key factor in metallic NP-induced toxicity, as well as modulation of cellular signaling involved in cell death, proliferation, and differentiation [28]. The results revealed that CeO_2 NPs had significant cytotoxic effects when compare with the control and that these effects were dose-dependent. Diaconeasa and co-authors [29] observed significant difference in cytotoxicity of CeO_2 NPs on cancer and normal cell lines. Since the cellular responses to cytotoxic agents differ, the cytotoxicity of CeO_2 NPs varied considerably between different cell lines [30] .

Cell viability assays showed that β-Carotene has the ability to decrease the proliferation of MCF-7, CAL-51, and HBL-100 cell lines, and these effects were increased by increasing the concentration. Figure 1-C shows that the percentage of viability peaked in lowest concentrations compared with highest concentration in the three cell lines. Therefore, the present study suggest that the high concentrations (150, 300, and 500 µg/ml) of β-carotene have higher cytotoxic effects than those of the low concentrations (5, 15, and 50 µg/ml). Another study showed a similar finding on LNcap cell line [7]. β-Carotene has multiple-potent biological effects, including its pro-oxidant role in stimulating ROS production in high concentrations [7], cell cycle arrest, DNA damage [31], and cell death[30]. Figure 1-D shows that the IC_{50} values of β-Carotene were 58.45, 165.30, and 82.47 µg/ml in CAL-51, MCF-7, and HBL-100 respectively. The statistical analysis showed a significant difference at $P \leq 0.05$ between these values, except the IC_{50} values of HBL-100 and CAL-51 cell lines. These results may be due to the nature of cell lines in relation to steroid hormones (estrogen, androgen, and HER2) [32, 33]. = β-Carotene leads to the inhibition of receptors of steroid hormones [34].Hence, the sensitivity of CAL-51 and HBL-100 cell lines is higher than that of MCF-7. Table 2 shows that the IC_{50} value of β-Carotene was significantly higher than that of CeO_2NPs on MCF-7 and HBL-100 cell lines, while no significant difference in IC_{50} values of these materials was observed on CAL-51 cell line at $P \leq 0.05$. This indicates that CeO_2 NPs was more toxic than β-Carotene on MCF-7 and HBL-51 cell lines.

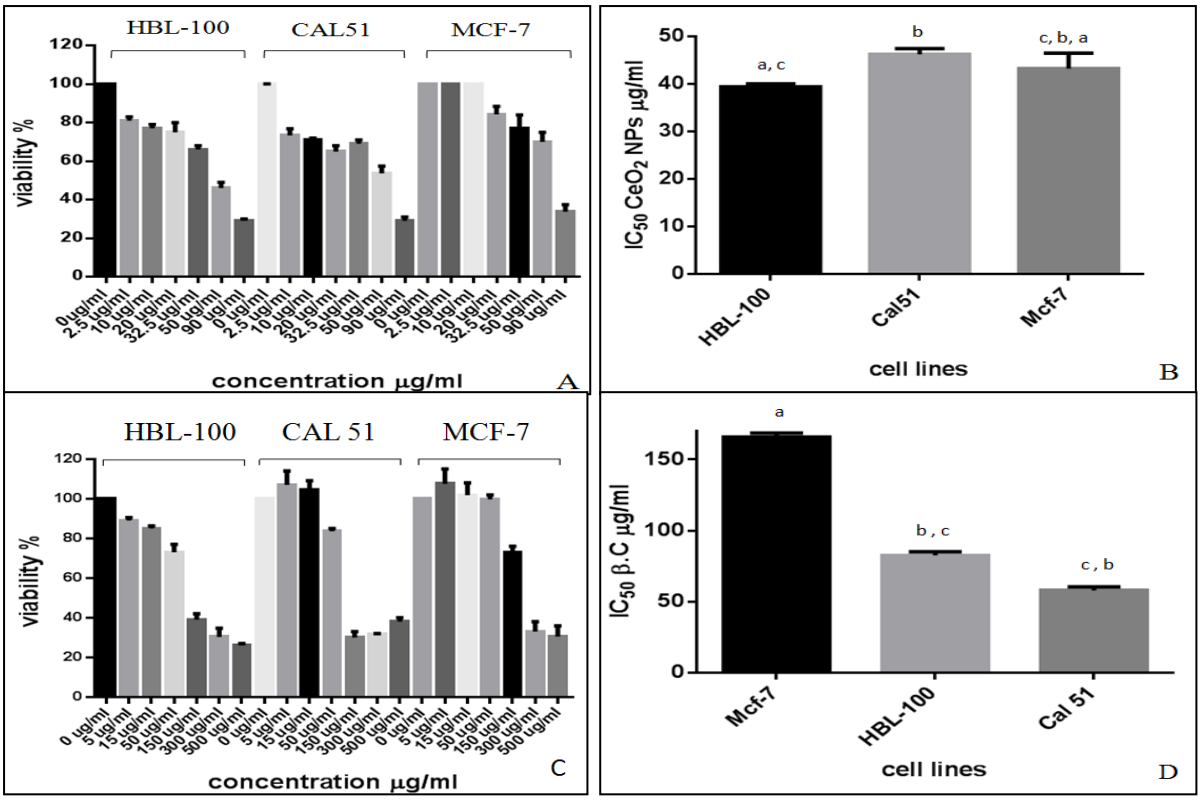


Figure 1-A-viability ratios of HBL-100 , Cal-51, and MCF-7 cell lines treated with CeO_2NPs (<5nm) for 72 h. **B-** IC_{50} of CeO_2 NPs (<5nm) of HBL-100, Cal-51, and MCF-7 cell lines. **C-** Viability ratios of HBL-100, Cal-51, and MCF-7 cell lines treated with β-Carotene for 72h. **D-** IC_{50} of β-Carotene for HBL-100, Cal-51, and MCF-7 cell lines .

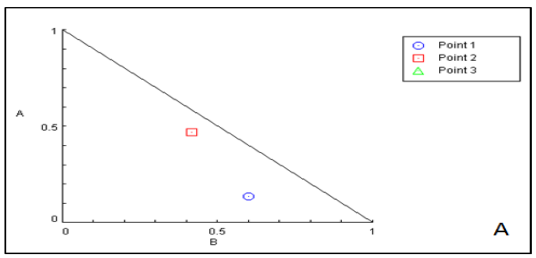
Table 2- A comparison between the IC_{50} values of CeO_2 NPs (<5nm) and β-Carotene against breast cancer and normal cell lines..

Cell lines	IC_{50} (mean±Std. Error)		Sig.
	CeO_2 NPs<5nm	β-Carotene	
MCF-7	43.24±2.89	165.3±8.45	*
HBL-100	39.82±2.12	82.48±12.61	*
CAL-51	46.21±5.40	58.4±0.76	

*The stars refer to significant differences.

The combined effects of CeO_2 NPs + β-Carotene

The results of Compusyan Isobologram analysis of the combination index (CI) for three concentrations of both CeO_2 NPs and β-Carotene for 72 h indicate synergistic effects with the second concentration (IC_{50}) in all cell lines. While, for the first concentration (20+50µg/ml), such effects were observed in MCF-7 cell line only, since the CI value was <1 (Tables 3, 4, 5 and Figure 2). In fact, each of β-carotene and CeO_2 NPs were found to generate ROS [35] and apoptosis [8, 36]. Therfore, the synergistic effects occurred upon combining those materials, as noticed by the increased levels of ROS and apoptosis. This finding might have occurred due to the association between cell cycle arrest induced by β-carotene [37] and the cytotoxic effect caused by CeO_2 NPs [7]. We suggest that the synergistic action was caused by independent behavior of each substance in cell inhibition.



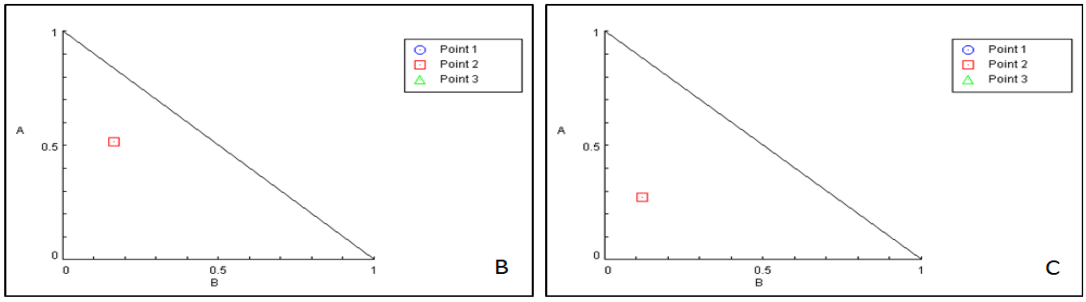


Figure 2-Combined effects of β-Carotene and CeO_2 NPs on cell lines after 72 h. A- MCF-7cell line, B- CAL-51 cell line , C- HBL-100 cell line. Point 1 is the combined effect of 20 µg/ml of CeO_2 NPs + 50 µg/ml of β-Carotene; point 2 is the combined effect of IC_{50} µg/ml of CeO_2 NPs + 50 µg/ml of β-Carotene; point 3 is the combined effect of 50 µg/ml of CeO_2 NPs + 300 µg/ml of β-Carotene

Table 3-The combined effects of β-Carotene (β-C) and CeO_2 NPs on MCF-7 cell line.

Dose CeO_2	Dose β-C	Effect	CI
20.0	50.0	0.6	0.73809
43.23	165.3	0.48	0.88704
50.0	300.0	0.735	26.2256

Table 4- Combined effects of β-Carotene (β-C) and CeO_2 NPs on CAL-51 cell line.

Dose CeO_2	Dose β-C	Effect	CI
20.0	50.0	0.195	3.71718
46.21	58.45	0.73	0.68297
50.0	300.0	0.645	2.08426

Table 5- Combined effects of β-Carotene (β-C) and CeO_2 NPs on HBL-100 cell line.

Dose CeO_2	Dose β-C	Effect	CI
20.0	50.0	0.165	4.32638
39.82	82.4	0.84	0.39344
50.0	300.0	0.494	4.03283

Morphological changes of cells

The microscopic examination of HBL-100, CAL-51, and MCF-7 cells showed different cytopathological changes after 72 h of treatment with the materials (CeO_2 NPs >5nm and β-carotene). The morphological changes were similar considerably between cell lines treated with the materials, as shown for HBL-100 (Figure 3 (A), CAL-51 (Figure 3 (E), and MCF-7 (Figure 4 (C) cells. The atrophy, along with the spherical and irregular shapes, observed in cells are among the hallmarks of the cytopathic effect. The cytoskeleton contributes significantly to the maintenance of cell shape. Previous studies showed that CeO_2 NPs can interfere with cytoskeleton organization [38]. Also, β-Carotene caused modifications in many cytoskeleton proteins, such as Lamin B1, Tubuline, Flotillin, and Cavalin2 [39]. Hence, any change in the organization of the cytoskeleton causes changes in the shape of the cell [40]. These events may interpret the cytomorphological changes in the cells. Other changes observed in this study include the large spaces that appeared in cell cultures, which are due to atrophy, shrinkage, and decomposition of cells (Figure 4 A, E). These ongoing morphological changes were brought about by the exposure to CeO_2 NPs and β-Carotene, accompanied with

karyopyknosis and hyperchromy of the nuclei (Figures 3 (B and F) and 4 (D)). Karyopyknosis is one of the characteristics of cell that is suffering apoptosis [24, 41]. Moreover, necrosis is another cell injury which was observed (Figure 3 F and 3 D) which might be attributed to free radicals (ROS) [42].

Cytoplasmic vacuolation was also observed in treated cells (Figures 3 C) and 4 Aand E) as a defensive step to isolate CeO_2 NPs and β-Carotene from the internal environment [43] . Nucleic fragmentation and apoptotic bodies were noticed in cells treated with β-Carotene, changes that indicated the induction of apoptosis [41]. In addition, multynucliated cells appeared in some cases (Figure 4 E). This may be due to the fusion of cells with each other or the impairment in cytokinesis [44].

The damage was further progressive when the cells were treated with the combination of the substances. The cells suffered from diverse cytomorphological changes (Figures 3 D and 4 B and F**)**.

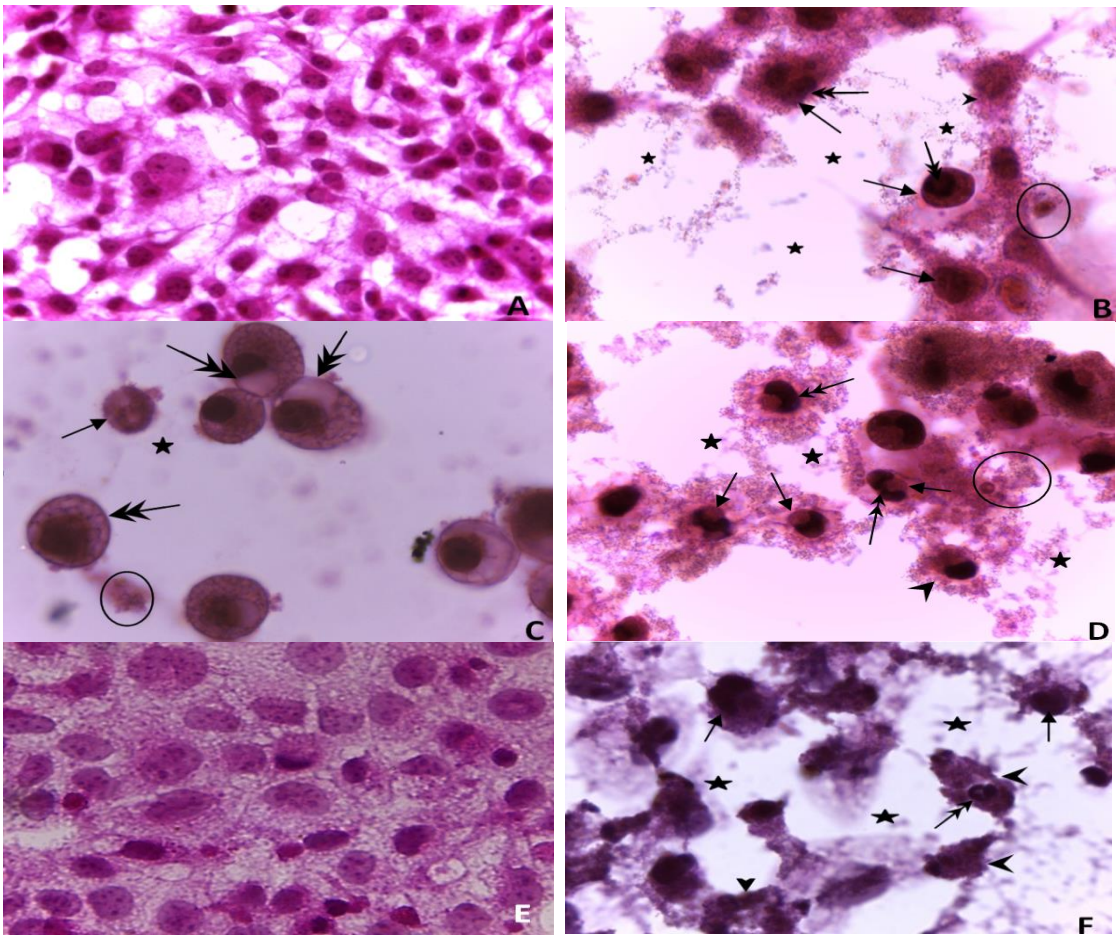


Figure 3- Morphology of HBL-100 and Cal-51cell lines stained with H&E - A: Untreated HBL-100 cells grown in monolayer with normal morphology 400x; B: treated cells at IC_{50} of CeO_2 NPs for 27h , showing changes in cell shape (arrow), necrosis (arrow head) , karyopyknosis (double arrow), decay of some cells (circle), and large spaces between cells (stars), 1000x; C: treated cells at IC_{50} of β.C., showing vaculation of cell cytoplasm (double arrow) , nuclei decomposition (arrow) , large spaces between cells (stars), and decomposition (circle) ; D: cells treated with the combination of IC_{50} (CeO_2 NPs + β.C.), showing cytoplasmic vacuolation (arrows) , kidney-shaped nucleus (dabble arrows), necrosis (head arrows) , large spaces between cells (stars), and decaying cells (circle). E: untreated Cal-51 cell line grown in monolayer with normal morphology, 1000x; F: treated cell line at IC_{50} of CeO_2 NPs, showing atrophy of hyperchromic cells (arrows) , necrosis (head arrows),nucleic fragmentation (double arrow), and large spaces between cells (stars).

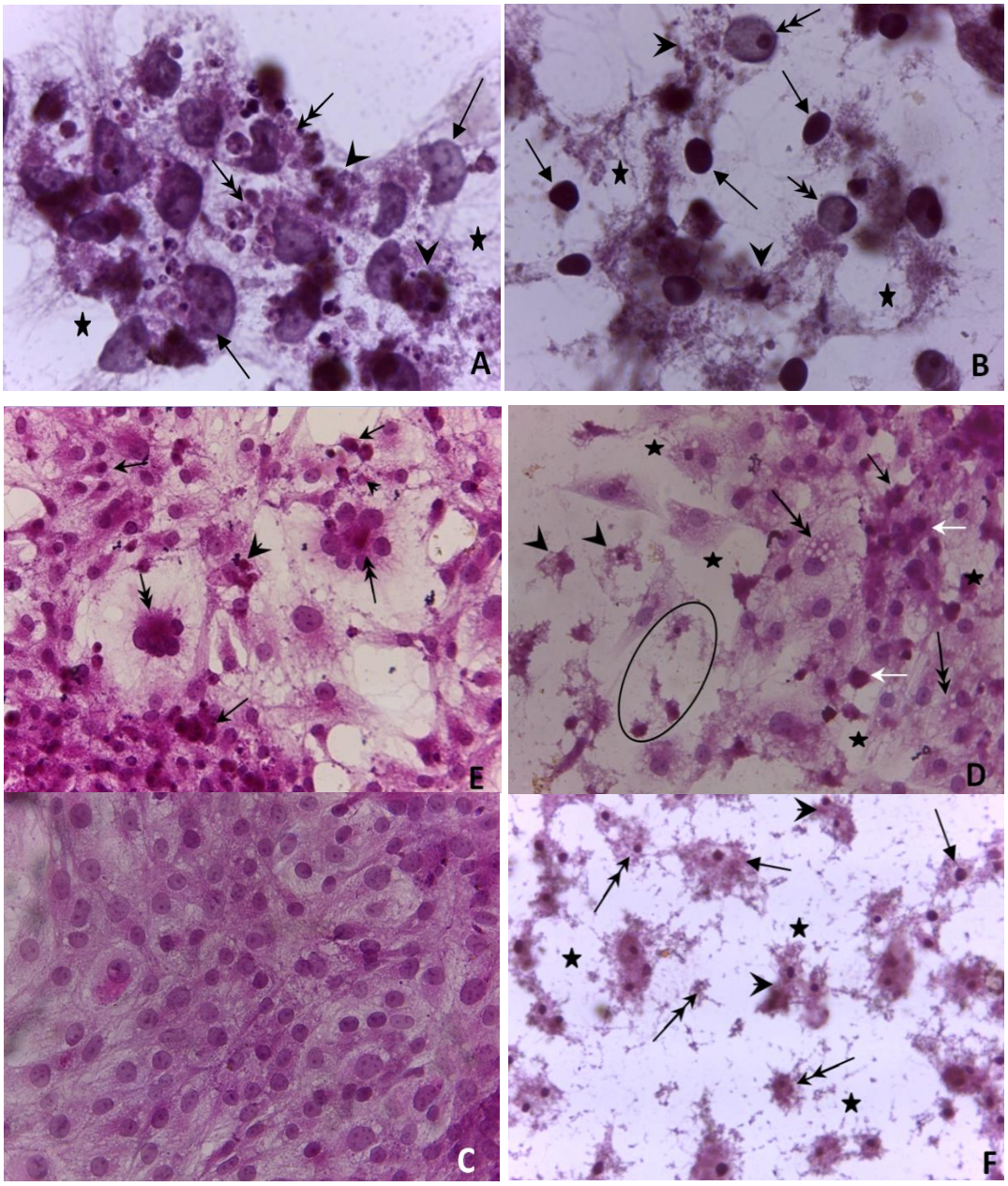


Figure 4-Morphology of HBL-100 and Cal-51cell lines stained with H&E. A: treated Cal-51 cell line at IC_{50} of β.C., showing changes to spherical-shape (arrow), necrosis (head arrow), formation of apoptotic bodies (double arrow), and presence of large spaces between cells (stars),1000x; B: cells treated with the combination of IC_{50} (CeO_2NPs+ β.C), showing conversion of cells to spherical-shape (double arrows), karyopyknosis (arrows) , necrosis (head arrows), and large spaces between cells (stare); C: untreated MCF-7 cell line grown in monolayer with normal morphology, 400x. D: treated cells at IC_{50} CeO_2 NPs, showing vacuolative degeneration (double arrows) , change in cell shape (black arrows) , necrosis (head arrows) , karyopyknosis, hyperchromy of some cells (white arrows), absence of large spaces between cells (stars), and cell decomposition (circle); E: treated cells at IC_{50} of β.C., showing karyopyknosis (black arrows) , multinucleated cells (double arrow), and necrosis (arrow head); F: treated cells with the combination of IC_{50} (CeO_2 NPs + β.C.), showing degradation (arrows), necrosis (head arrows) , nuclei degradation, hyperchromy (arrows), large spaces between cells (stars), and decomposition (double arrow).

Cell death

The AO/EB assay was used to observe the differential uptake of fluorescent DNA-binding stain AO/EB [15]. The untreated cells were stained with green color, indicating intact cells (Figures 5 A, E and 6 C), while some of the cells treated with the IC_{50} of CeO_2 NPs, β-Carotene, and their combination for 72 h were stained with yellow or red color, which indicates cell death (Figures 5 (B, C, F) and 6 (A,B, D, E,F)). The cytomorphology that processed by AO/EB protocol is at least shown to, if not ensure than, mechanism of materials, cytotoxic effect on cell lines,. In addition, the results confirm necrosis incidence in all cell lines. The staining with red indicates that the necrotic and apoptotic cells suffered from a defect in permeability, which caused the penetration of EB through the plasma membrane and nuclear envelope, consequently leading to their contact with the nuclear material of the cell [45]. The yellow cells are in an ongoing pathway to death, appearing as pro-apoptotic or pro-necrotic cells [13]. Untreated cells were stained with green color of AO stain, an indication of the integrity of their membranes [45].

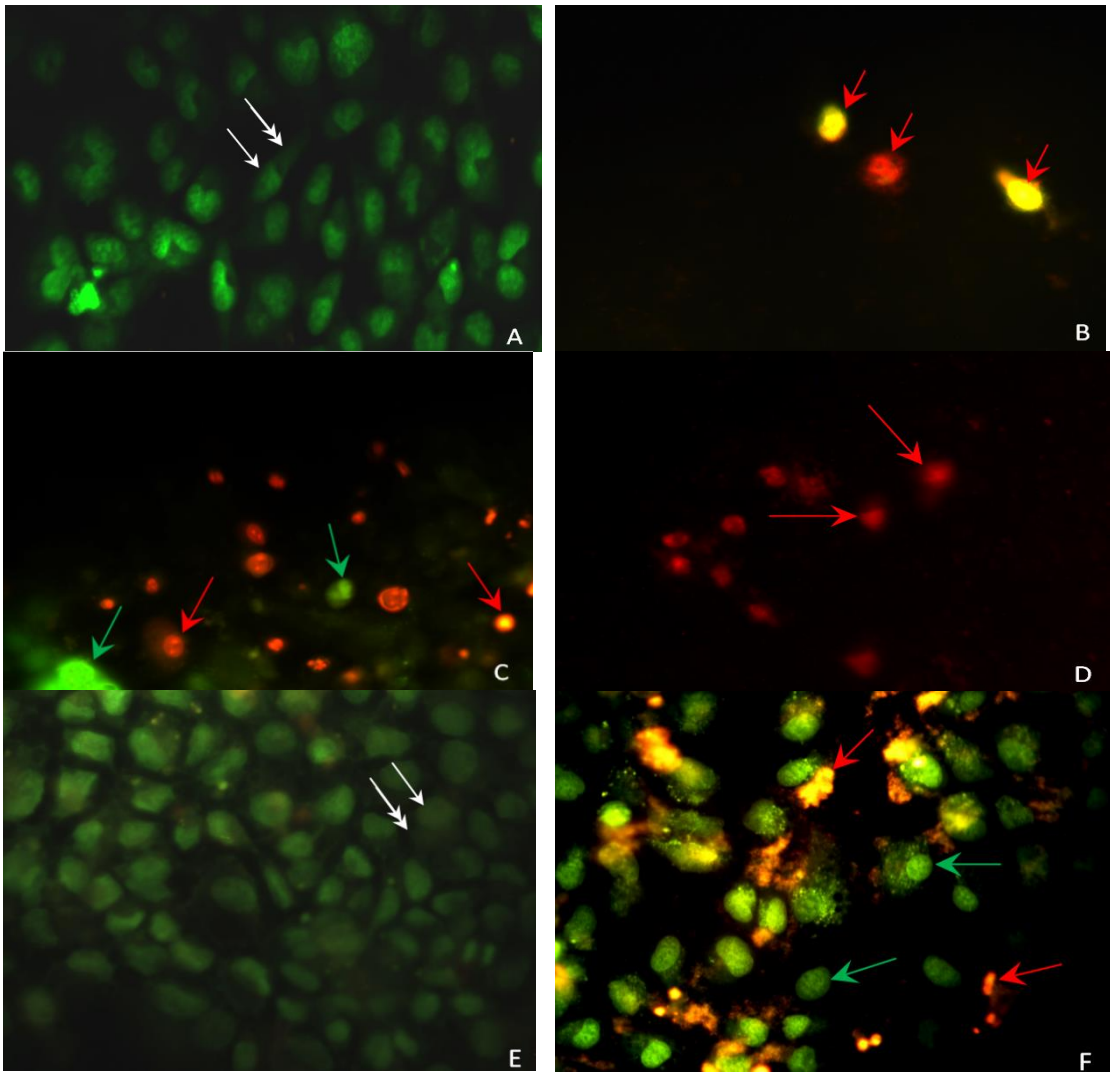


Figure 5- AO/EB staining of cells. A- untreated HBL-100 cells; B- cells treated with IC_{50} of CeO_2 NPs; C - cells treated with IC_{50} of β.C; D- cells treated with the combination of IC_{50} (CeO_2 NPs & β.C.). E- untreated CAL-51 cells; F- treated cells at IC_{50} of CeO_2 NPs. The arrow refers to the nucleus the double arrow refers to the cytoplasm , the green arrow refers to the living cells , and the red arrow refers to the dead cells (400x magnification).

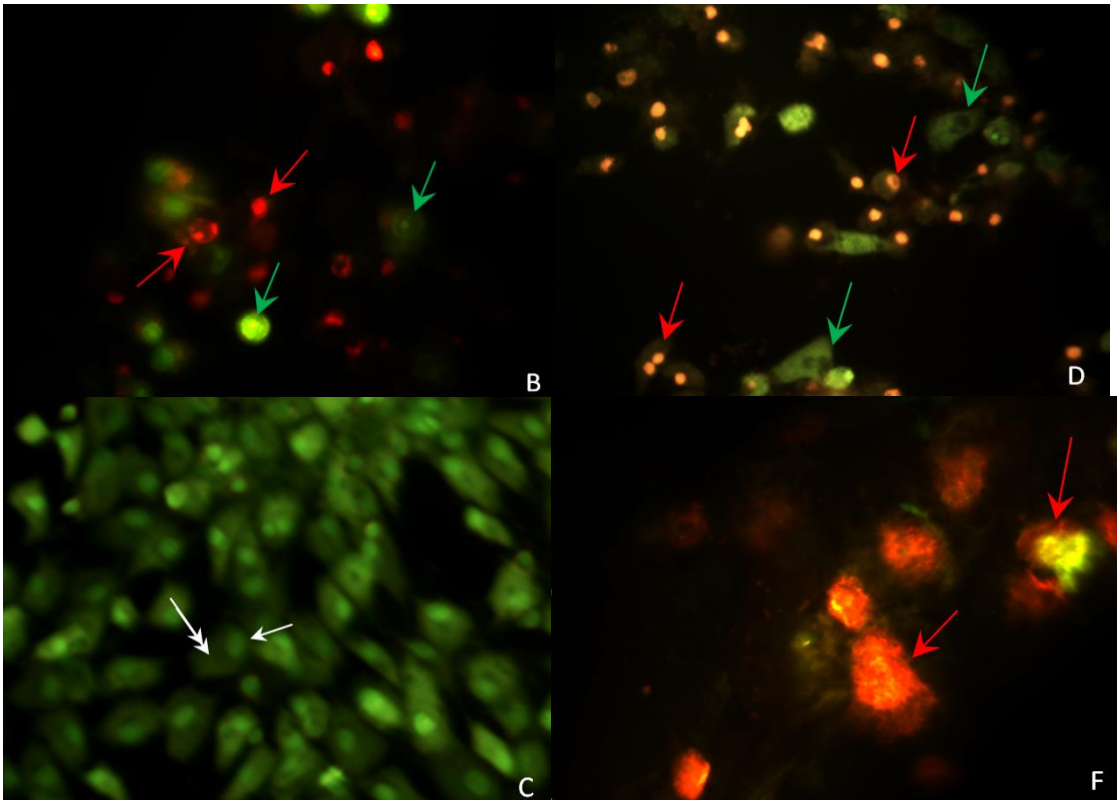


Figure 6- AO/EB staining of cells A- Treated Cal-51 cells with IC_{50} of β.C. 400x; B- Cells treated with the combination of IC_{50} (CeO_2NPs + β.C.); C- Untreated MCF-7 cells; D- cells treated with the IC_{50} of CeO_2 NPs; E- Cells treated with the IC_{50} of β.C.; F- Cells treated with the combination of IC_{50} (CeO_2 & β-C), 1000x. The arrow refers to the nucleus , the double arrow refers to the cytoplasm , the green arrow refers to the living cells , the red arrow refers to the dead cells.

Figure (7 A, B, C) shows the DNA fragmentation in MCF-7, CAL-51, and HBL-100 cells treated with CeO_2 NPs and its combination with β-Carotene. Our findings demonstrate that there was DNA cleavage at ≤ 100 bp in all treated cells. In addition, there was a smear on the agarose gel for all treated cells. The DNA fragmentation did not appear in untreated cells and cells treated with β-Carotene. Moreover, the results indicate that the CeO_2 NPs overlapped with the DNA and altered the metabolic function, then leading to defects in cellular components [46]. The small-sized CeO_2 NPs induced primary DNA lesions. This DNA damage was reported to be induced by oxidative stress [24]. These results suggest DNA damage that indicates the induction of apoptosis.

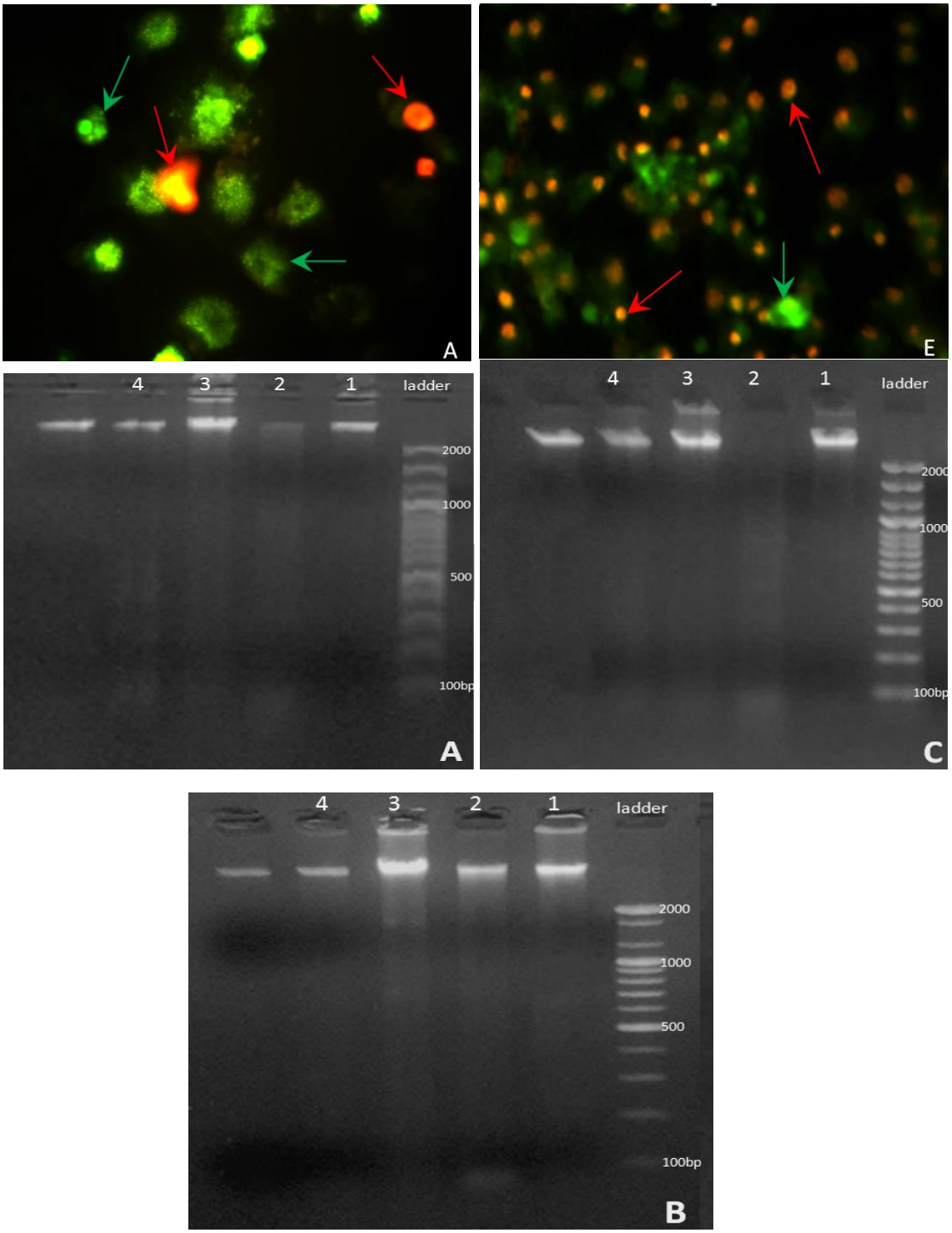


Figure 7- Run of DNA of cell lines on agarose gel 1.5 % at 85 volts for 55 min. A- HBL-100. B- CAL-51 and C-MCF-7. 1-Untreated cells; 2 –Treated cells at IC_{50} of CeO_2 NPs; 3- Treated

Figure 8 (A and D) shows that the level of caspase-8 gene expression in HBL-100 cells was significantly higher in cells treated with β-Carotene than in cells treated with CeO_2 NPs. In contrast, CAL-51 cells treated with CeO_2NPs showed significantly higher caspase-8 gene expression at $P \leq 0.05$. There was no significant difference in caspase-9 and p53 gene expression in all treated cells at $P \leq 0.05$. This indicates that apoptosis was carried out via the extrinsic death domain. The two substances (CeO_2NPs and β-Carotene) had the ability to cause cell death and DNA damage in cell lines and induced the external pathway of apoptosis [47].

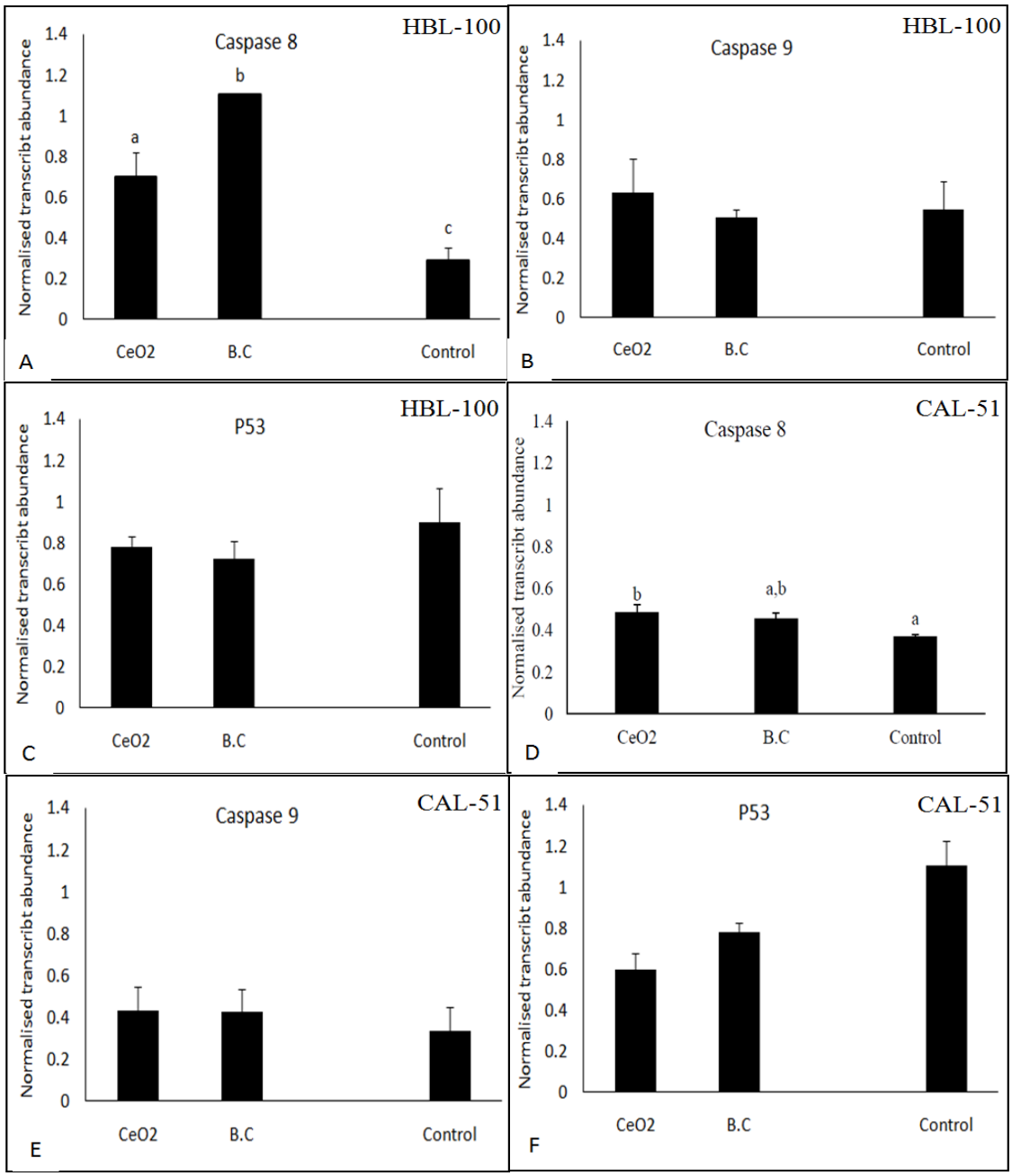


Figure 8- Gene expression of caspase8, caspase9, p53 in HBL-100 and CAL-51 cells treated with the IC_{50} of CeO_2 NPs (CeO_2), β-Carotene (β-C), and untreated cells (control). A- The increase of caspase 8 expression in HBL-100 cells treated with β-C compared to untreated cells and cells treated with CeO_2 NPs at $P \leq 0.05$. D- The increase of caspase 8 gene expression in CAL-51 cells treated with IC_{50} of CeO_2 NPs compared to untreated cells at $P \leq 0.05$. B and E- No significant deference in caspase 9 expression in the two cell lines; C and F- No significant difference in p53 expression in the two cell lines

In conclusion, CeO_2 NPs have more toxic effects on MCF-7, CAL-51, and HBL-100 cells than β-Carotene, while both substances have no selectivity on cell lines. Our findings suggest that the genotoxic effects of both materials occur through the apoptosis and necrosis pathways.

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