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Curcumin intercalated layered double hydroxide nanohybrid as potential drug delivery system for effective photodynamic therapy in human breast cancer cells

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Abstract

Curcumin as a natural phenolic compound is high potent anticancer agent against many different types of cancer. Recent studies show that curcumin can be used as a photosensitizer in photodynamic therapy for cancer treatment. However, the major disadvantage of curcumin is its poor aqueous solubility. To improve its applicability in cancer therapy we intercalated curcumin into layered double hydroxide (LDH) with the co-precipitation method and used as nanohybrdie photosensitizer in photodynamic therapy of human breast cancer cells. Powder X-ray diffraction (XRD), TEM and SEM microscopic analysis indicate that curcumin is stabilized in the host interlayer. According to the spectroscopic results the water solubility and dispercity of intercalated curcumin increased and loading amount of curcumin in LDH is about 50%. The photodynamic effect of curcumin and curcumin-LDH nanohybrid was studied on MDA-MB-123 human breast cancer cell line. Optimization of incubation time with free curcumin and curcumin-LDH nanohybrid as the most effective parameter was investigated. The optimum irradiation time of blue LED on photodynamic therapy was determined for both free curcumin and curcumin-LDH nanohybrid. Cell viability studies revealed that the nanohybrid curcumin-LDH were able to show more effective photodynamic effect on the cancer cells as compared to free curcumin. The results suggest that bio compatible layered double hydroxide can be used as the basis of a tunable curcumin delivery carrier for photodynamic therapy in breast cancer treatment.

Keywords: Layered Double Hydroxide (LDH), Curcumin nanohybrid, Photodynamic therapy, Breast cancer, Drug delivery

Introduction

Breast cancer is known as the most common cancer for women in the world. Developed countries have higher population (approximately 25% of all female malignancies) of women with this cancer. Breast cancer is the second leading cause of cancer-related death among females in the world [1]. Recently, various researches have been focused to achieve the effective therapy method for breast cancer[2,3]. Photodynamic therapy (PDT) is a novel and useful technique for treatment of a variety of diseases and cancers. The principle of PDT is illuminating a photosensitizer with light at specific wavelength to producing reactive oxygen species (ROS), especially singlet oxygen, in tumor cells. The tumor cells will be destroyed by apoptosis and/or necrosis induced by ROS and finally the local treatment with minimum invasion will be achieved[4,5]. The photosensitizer plays a crucial performance in an efficient PDT[6,7]. Curcumin (1, 7-bis (4-hydroxy 3-methoxy phenyl)-1, 6-heptadiene-3, 5-Dione), a natural polyphenolic pigment from Curcuma longa (turmeric) rhizomes, is a well-known anti-carcinogenic, wound-healing and anti-inflammatory agent [8–10]. Poor solubility and bioavailability of curcumin in aqueous solution limited the curcumin applications in medical and clinical applications [11]. However the effective delivery of curcumin for cancer treatment is very interesting for various scientists and needs to be studied. Many studies have been reported on modified curcumin with different functionalities for evaluating its anticancer activities [12]. Among the published researches, the encapsulation of curcumin in liposomes, polymeric micelles, intercalation in cyclodextrines and other methods are reported and used for increasing the curcumin solubility and bioavailability[13,14].

Layered double hydroxides (LDHs), also called anionic nano-clays, are an important class of layered inorganic materials that make a network of divalent and trivalent metal cations cross-linked with hydroxide anions and contain charge balancing interlayer anion[15]. In recent studies, LDHs have been used as drug delivery system due to their good biocompatibility,

low cytotoxicity, unique anionic exchange property and pH-controlled release property in acidic environments [16,17]. In some studies the role of curcumin as a photosensitizer in antimicrobial Photodynamic therapy was investigated [18,19]. Up to now, there are few studies for intercalating curcumin in nanoparticles as photosensitizer. Also using of LDHs as nano-drug delivery system for PDT have recently developed [20,21] but there is no any report on curcumin intercalation in LDH as photosensitizer in PDT.

In this work the curcumin intercalated LDH materials was prepared, characterized and evaluated for curcumin delivery into MDA-MB-231 cell lines as photosensitizer in PDT. . The results show that curcumin intercalated LDHs nanohybrid has more photodynamic effect in comparison to free curcumin. It can be suggested that curcumin-LDH nanohybrid can be work as potential drug delivery system for PDT on MDA-MB-231 breast cancer cell line.

Materials and Methods

Materials

Curcumin ((1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione ($C_{21}H_{20}O_6$) , 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), tryphan blue solution 0.4% and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS), phosphate buffered saline (PBS) and antibiotics were purchased from Gibco (Gibco BRL). Dulbecco's Modified Eagle Medium (DMEM) was purchased from Invitrogen (Invitrogen, Carlsbad, California, US). All the other reagents were obtained from Merck. Deionized (D.I.) water was used for the entire experiments.

Synthesis of LDH

An aqueous solution (100 ml) containing NaOH (0.2 mol) was added dropwise to a solution (160 ml) containing Mg $(NO_3)_2 \cdot 6H_2O$ (0.006 mol) and $Al(NO_3)_3 \cdot 9H_2O$(0.002 mol) under nitrogen atmosphere with vigorous stirring until the final pH 10. The resulting slurry was transferred in Teflon lined autoclave for hydrothermal calcination at 400 ˚C for 2 h and then was cooled and filtered, washed with fresh and CO_2 free de-ionized water until the pH 7 and finally dried in vacuum at room temperature for 12 h giving the product.

Intercalation Curcumin into LDHs

An aqueous solution of (100 mL) NaOH and Curcumin (0.0003 M) was added dropwise to a solution (250 mL) containing Mg $(NO_3)_2 \cdot 6H_2O$ (0.006 M) and $Al(NO_3)_3 \cdot 9H_2O$ (0.002 M) (molar ratio Mg/Al=3.0) under nitrogen atmosphere with vigorous stirring until the final pH of 10. The resulting slurry was transferred in Teflon lined autoclave for hydrothermal calcination at 400 ˚C for 2 h and then was cooled and filtered, washed with fresh and CO_2 free de-ionized water until the pH 7 and then dried in vacuum at room temperature for 12 h giving the product (Curcumin intercalated LDH nanohybrid).

Loading efficiency and solubility study of curcumin

In order to determine the loading efficiency of curcumin (%) in curcumin-LDH-NPs, UV–vis spectrophotometric studies (using Cary 100 spectrophotometer) at 430 nm were carried out. A standard curve in the range of 0–20 μg/ml curcumin was plotted. The curcumin content present in curcumin-LDH-NPs was calculated as loading efficiency using Eq. (1).

$$Loading\ efficiency(\%) = \frac{[curcumin]_{total} - [curcumin]_{free}}{[curcumin]_{total}} \times 100 \qquad (1)$$

Aqueous solubility of curcumin and curumin-LDH nanohybrid was determined by dissolving separately in 0.01 M of PBS in pH 7.4. The nanohybride increased the aqueous solubility of curcumin up to 6.15. For this purpose, the certain amount of both curcumin and curcuin nanohybride was used (100 μg/ml). The sample solution stirred for 5 min. Then the certain clear solution amount of each sample transferred in quartz cell for measuring maximum absorbance of solution by considering A_{max} . Also, we determined baseline for each graph (curcumin and curcumin-LDH nanohybrid) then we determined the difference between baseline and maximum absorption in each graph for eliminate baseline effect. The enhancement of solubility of curcumin in nanohybrid could be calculated as $A_{max\text{-}curcumin\text{-}nanohybrid}/A_{max\ curcumin}$.

Characterization of curcumin-LDH nanohybrid

The absorption spectra were measured using Cary 100 UV/Vis spectrophotometer, equipped with quartz cuvettes. The Fluorescence spectra measurements were performed on a Cary Eclipse fluorescence spectrophotometer equipped with a thermostatically controlled cell holder at ambient temperature. The monochromatic slits were set at 5 nm for excitation and emission, respectively, to reduce the intensity of the signal depending on the experiment. Powder X-ray diffraction (XRD) patterns were obtained by Rigaku Miniflex X-ray diffractometer using CuKα radiation (λ=0.154 nm). The morphology of samples was examined using scanning electron microscope (SEM). The surface morphology of samples was analyzed using a transmission electron microscope (TEM).

Cell line and culture

Human breast cancer cell line, MDA-MB-231, was obtained from the Institute of Pasture, Tehran, Iran. These cells were grown in DMEM medium supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml of streptomycin then incubated in a humidified incubator

containing 5% CO_2 at 37 ˚C. For the experiments, the cells were removed by trypsinizing (trypsin 0.025%, EDTA 0.02%) and washed with phosphate-buffered saline (PBS).

Effect of different incubation time with curcumin and curcumin-LDH nanohybrid on human breast cancer Cells

We designed the experiment for determining the optimum incubation time of curcumin and curcumin-LDH nanohybrid on cells via counting live and dead cells after specific times. About 1×10^4 MDA-MB-231 cells in culture medium were seeded in petri dishes and incubated overnight at 37 ˚C with 5 % CO_2. After 24 h, cells were incubated in a fresh growth medium containing different concentrations of curcumin and curcumin-LDH nanohybrid (0, 10, 25 μg/ml). After a specified time period of incubation (1, 4, 24, and 48 h) the growth medium containing curcumin and curcumin-LDH nanohybrid was removed and cells were washed with PBS. One plate of treated cells was irradiated with a blue LED source (465 nm; power density: 34 mW/cm^2) and another was kept in the dark, outside the incubator, for 30 min. Cell viability was determined by tryphan blue exclusion method. All experiments were repeated three times.

Effect of different irradiation time on human breast cancer Cells

For determining the effect of different irradiation time, MDA-MB-231 cells at a density of 1×10^4 cells/well were seeded in 96-well flat-bottomed micro titer plates (Jet Biofil Cat. No. TCP011096). After 24 h, the cells were incubated in a fresh growth medium containing different concentrations of curcumin and curcumin-LDH nanohybrid (0, 10, 25 μg/ml). After a further incubation of 24 h, the cells were washed with PBS. One plate of treated cells was irradiated with a blue LED source (465 nm; power density: 34 mW/cm^2) and another was kept in the dark, outside the incubator, for specified time periods (5, 10, 15, 20 and 30 min). All irradiations were performed at room temperature (25 ˚C). The colorimetric 3-(4, 5-

dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay was used to determine the cell viability. All experiments were repeated three times.

In vitro photodynamic assay

MDA-MB-231 cells were grown in medium culture cell and after reaching 80~90% confluence; the MDA-MB-231 cells were washed with PBS, afterwards detached from the flask by addition of 1.0 mL of 0.25% trypsin for 1−3 min at 37 °C. MDA-MB-231 cells (1×10^4 cells/well) were seeded into two 96-well plates. The cells were then treated with curcumin and curcumin-LDH nanohybrid at different concentrations. After a further incubation of 24 h, one plate was irradiated with a blue LED source (465 nm; power density: 34 mW/cm^2) and another was kept in the dark for 30 min.

To determine cytotoxicity of void LDH (drug free), additional groups of cells were incubated with different concentrations of LDH (0–100 μg/ml) and cell viability was measured in presence and absence of LED irradiation. All irradiations were performed at room temperature (25 ˚C). The MTT assay was used to determine the cell viability. All experiments were repeated three times

Cell viability

Cell survival was determined by MTT colorimetric assay. In brief, culture medium was removed and cells were incubated in medium with 0.5 mg/ml of 2-(4, 5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide (MTT) for 4 h at 37 ˚C. The resulting formazan crystals were dissolved with 100 μL dimethyl sulphoxide (DMSO) and shaking for 15 min. The absorbance was measured at 540 nm using an ELISA reader (Hyperion, Inc., FL, U.S.A.).

Statistical analysis

Statistical analysis was performed with Student's t-test (two tailed). All values are expressed as means ± SD. Results are expressed as with n denoting the number of experiments. $P < 0.05$ was considered as statistically significant.

Results

Physicochemical characterization of curcumin-LDH complex

Curcumin loading efficiency was found to be about 50%. Solubility of curcumin and curcumin-LDH nanohybrid was also determined by dissolving both curcumin and curcumin-LDH nanohybrid into the aqueous solution and compared (Fig. 1A). It revealed that curcumin-LDH nanohybrid was completely dissolved and a clear orange well dispersed liquid could be seen. The solubility of curcumin in nanohybrid was increased 6.15 time in compare to alone curcumin in solution.

Figure 1B, shows the absorption spectra of curcumin loaded in LDH (curcumin-LDH complex), free curcumin and LDH in aqueous solution. The absorption spectra of curcumin-LDH showed the higher absorbance peak (512 nm) with red shift as compared to free curcumin (430 nm). As seen in figure 1B, fluorescence spectra of curcumin-LDH nanohybrid showed a same pattern in the fluorescence peak with reduction in intensity as compared to free curcumin. The fluorescence intensity of intercalated curcumin quenched in comparison with free curcumin fluorescence spectra. The XRD, SEM and TEM results approved the spectrophotometric results regarding to intercalation of curcumin in the layers of LDH structure.

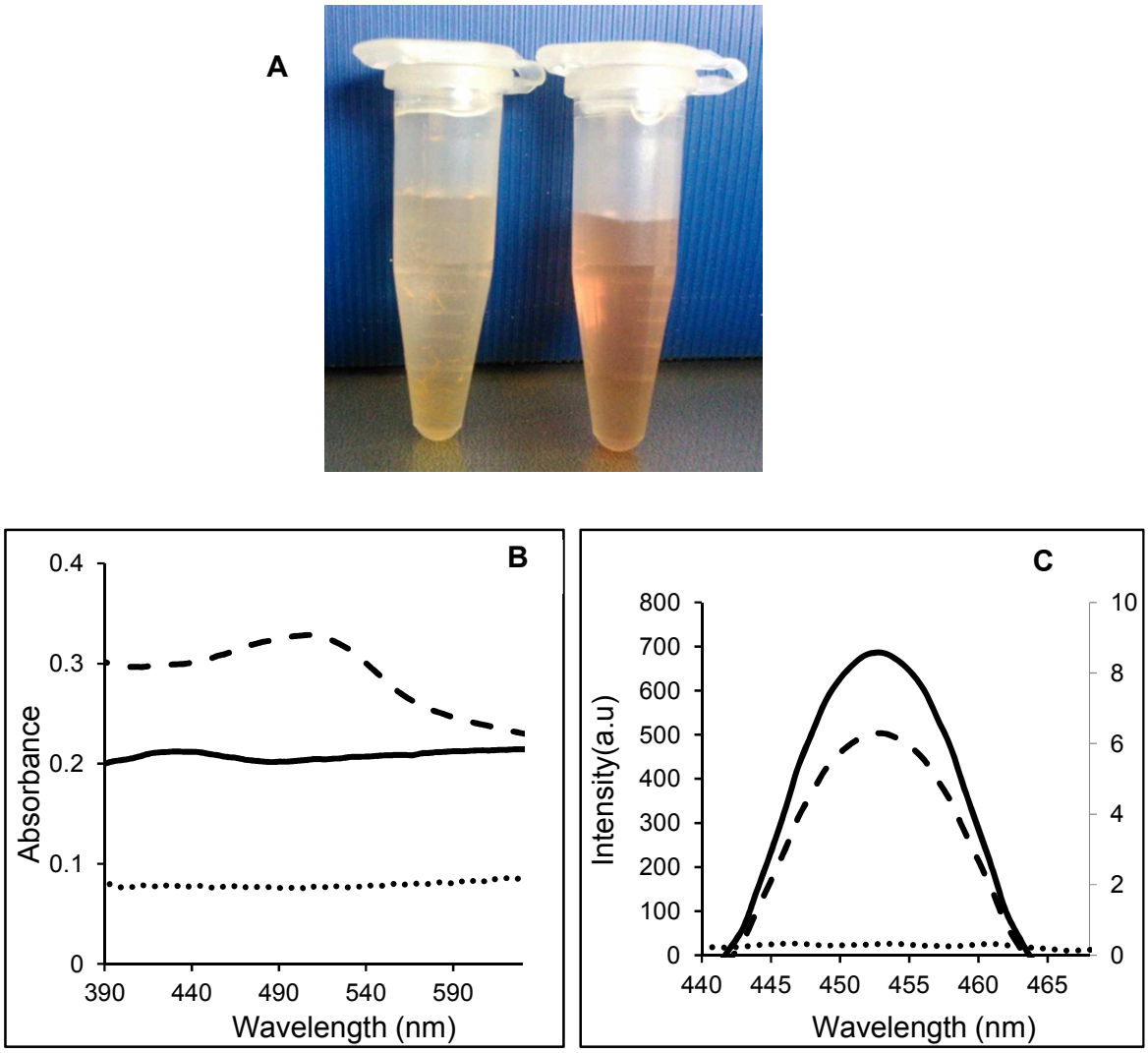
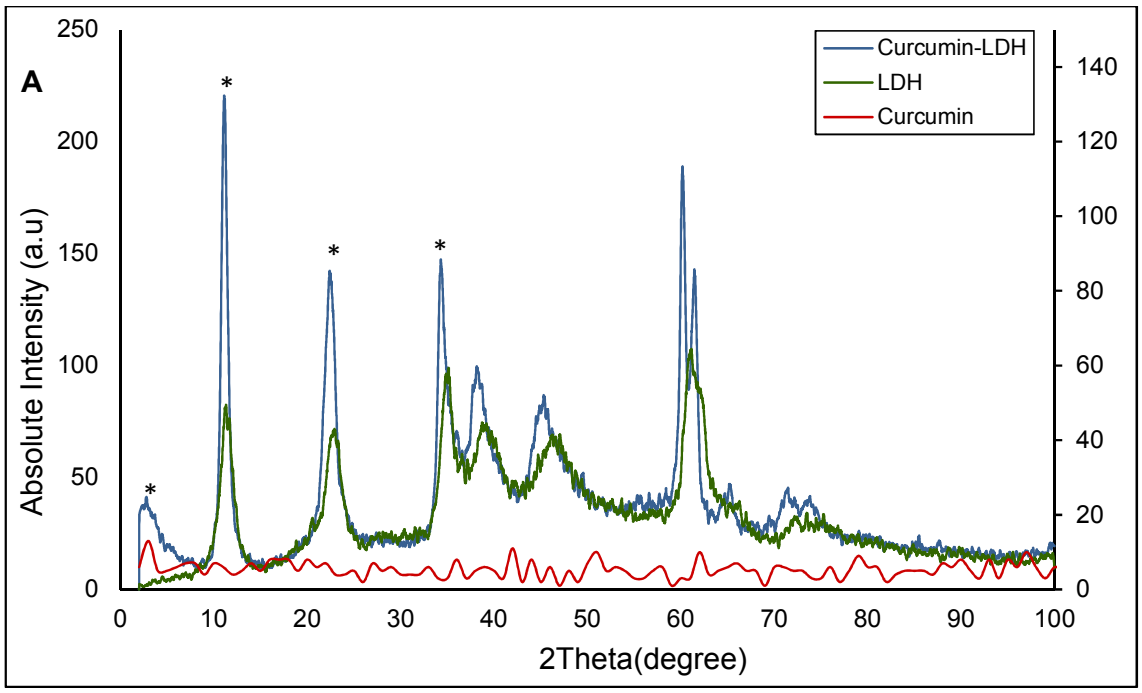


Figure 1. (A) The comparative solubility of curcumin and curcumin-LDH nanohybrid in PBS [(left) curcumin & (right) curcumin-LDH nanohybrid in PBS (0.01 M, pH 7.4)]; (B) Absorbance spectra and (C) fluorescence emission spectra of free curcumin (solid line), curcumin–LDH complex (dashed line) and LDH(dotted line) in aqueous solution.

Structural and Morphological Characterization

Curcumin intercalation into the interlayer region of LDH was confirmed by X-ray diffraction (XRD) analysis. The XRD patterns of curcumin-Mg_2Al-LDH samples are shown in Figure 2 A. In each case, typical LDH XRD pattern exhibits the characteristic reflections of the LDH layered structure with a series of peaks related to the stacked layers; strong lines

at low angle (marked by *), indicating the guests have been successfully intercalated into the LDH gallery to produce a supramolecular structure [22]. Also left shift of these peaks in curcumin-LDH XRD pattern is due to intercalation of curcumin and expanding layers. SEM (Figure 2 B) and TEM (Figure 2 C) images show that the sample of curcumin-LDH nanohybrid possesses uniform plate-like morphology with particle size ranging in 70-90 nm.



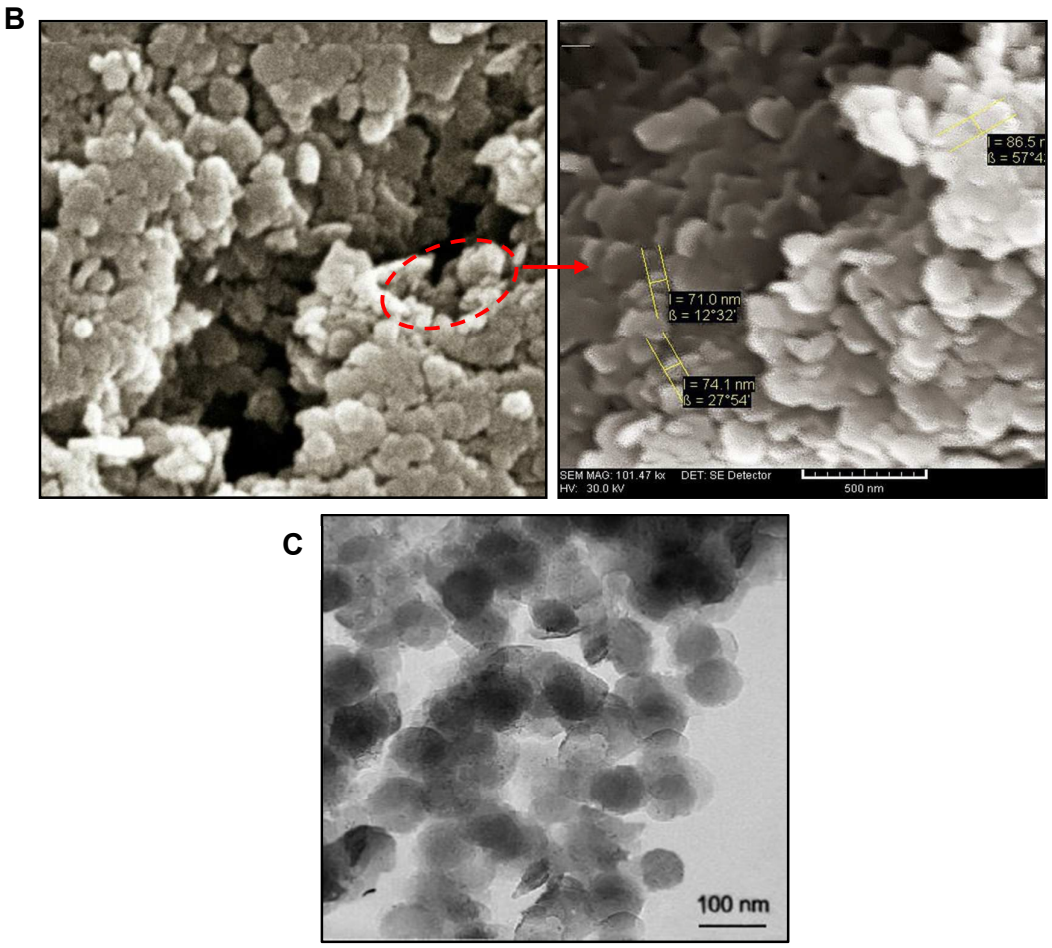


Figure 2. (A) The XRD patterns of Curcumin-LDH and free LDH ((*) indicate strong lines at low angle) (B) SEM image of the curcumin-LDH sample (C) TEM image of the curcumin-LDH nanohybrid.

Cytotoxicity of void LDH nanoparticle

In order to determine the toxicity of LDH used for curcumin loading, the effect of LDH (void) on cell viability was studied. Viability of cells treated with different concentration of LDH (up to 100 µg/ml, for 24 h) did not change significantly (figure 3). The sample of LDH does not show PDT effect as well as cytotoxicity, demonstrating its biocompatibility as reported previously [23].

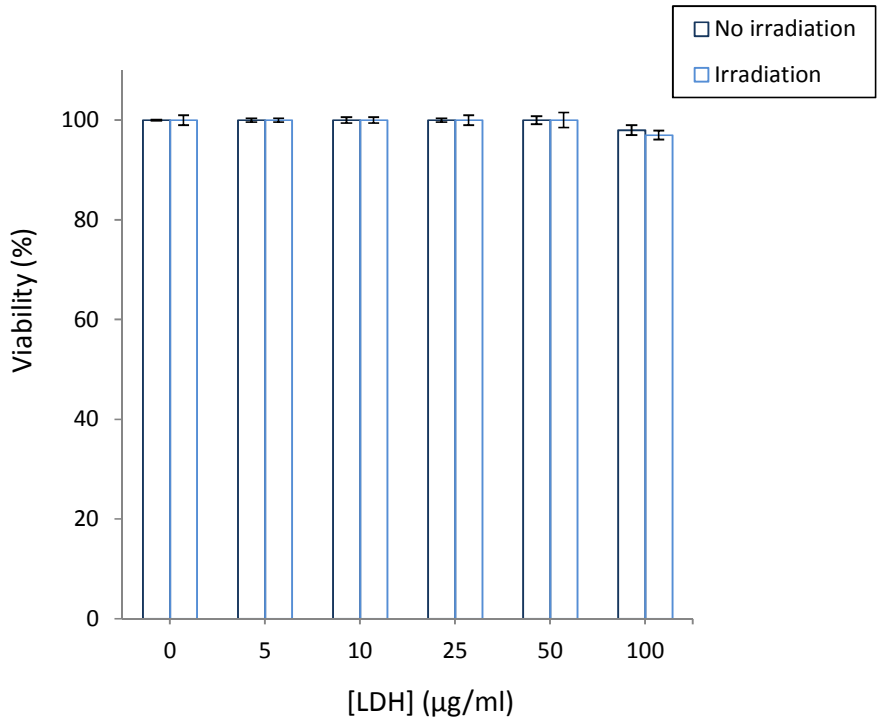
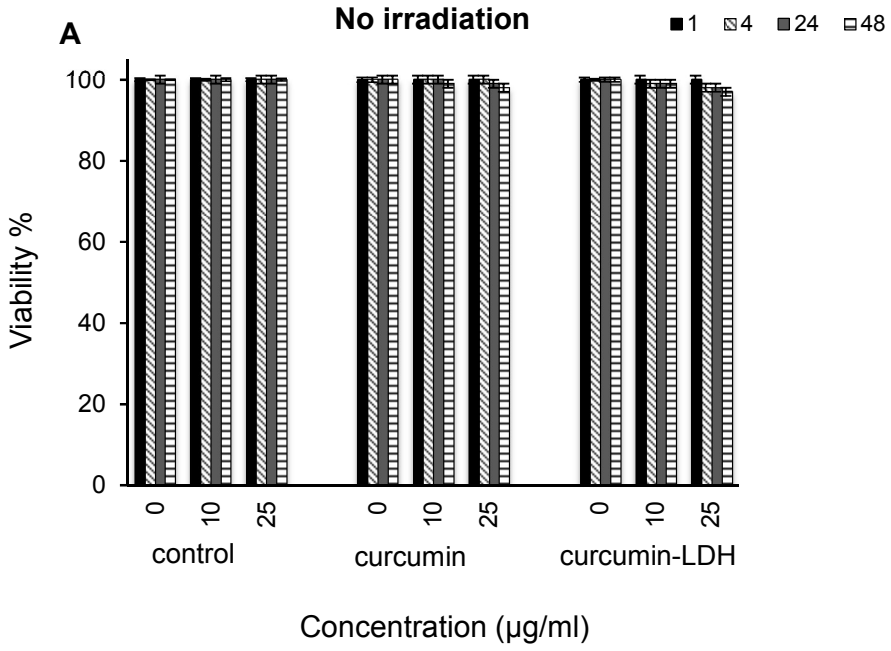


Figure 3. Viability of MDA-MB-231 breast cancer cells treated with indicated concentrations of LDH for 24 h and then one group kept in dark and another exposed to blue LED for 30 min. The results are expressed as the mean ± SD (n = 3).

Pre-incubation time dependent of curcumin-LDH nano hybrid phototoxicity on human breast cancer cells

The effect of various pre-incubation times with curcumin and curcumin-LDH nano hybrid on viability of cells was investigated with and without exposure to light. As seen in figure 4, no significant change in viability was seen for 1 and 4 h of incubation. Dark toxicity of curcumin-LDH nano hybrid slightly increased with increasing incubation times (24 and 48 h). The 98% viability was observed in cells pretreated with 25 µg/ml curcumin-LDH nano hybrid for 24 h. Exposure of these cells to blue LED light (~34 mW/cm^2) decrease the

viability to 61%. On the other hand, increasing incubation time did not have a significant influence on the viability of cells pretreated with a similar concentration of free curcumin. The results of 48 h incubation is similar to 24 h. The viability of treated cells with 25 μg/ml curcumin-LDH nano hybrid after 48 h was 97%. Exposure of these cells to light (~34 mW/cm^2) reduces the cell viability to 54%. Blank test shows that the irradiation imposes no influence on the cell viability.



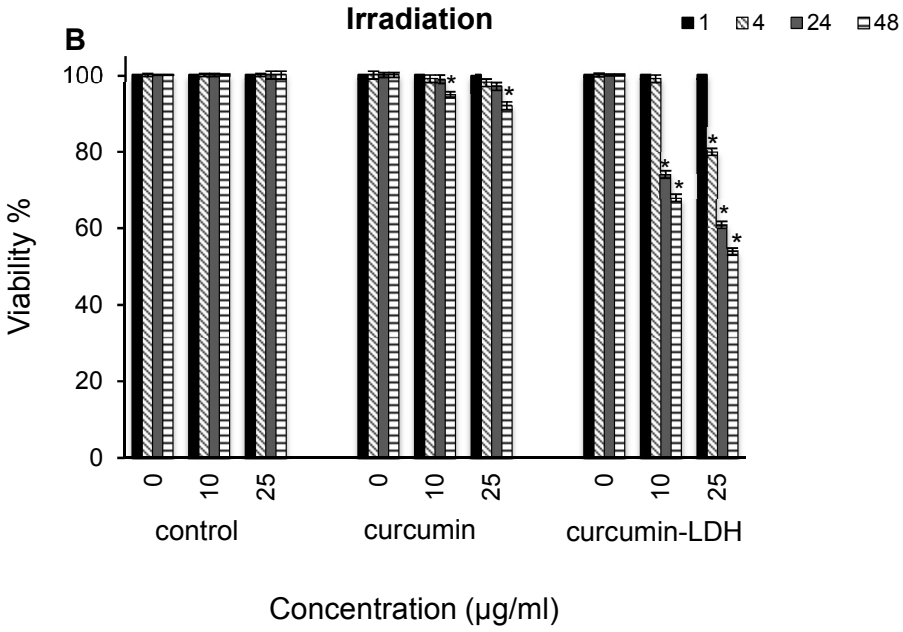


Figure 4. Viability of cells treated with 0, 10 and 25 µg/ml of free curcumin and equivalent concentration of curcumin–LDH complex for 1 , 4, 24 and 48 h and then kept in dark (A) and or exposed to blue LED (B) for 30 min. Viability of cells after different treatments was compared with untreated controls. The results are expressed as the mean ± SD (n = 3, *P < 0.05 compared with control group).

Effect of different irradiation time on human breast cancer Cells

The effect of light dose on cell death pretreated with either free or curcumin–LDH monohybrid were determined. The results presented in figure 5 show that there is no any significant difference in the viability of the cells treated with free curcumin and curcumin-LDH nanohybrid kept in dark. However, the viability of treated cells with 25µg/ml curcumin-LDH nanohybrid and irradiated was decreased gradually in the light dose dependent manner. The viability of the cells (curcumin-LDH nano hybrid treatment) exposed to 10, 15, 20 and 30 min irradiation, were 90%, 83%, 70% and 61%, respectively. The viability of free curcumin treated cells that were exposed to blue LED light was not changed

significantly (figure 5). The blank test shows that the various irradiation times impose no influence on the cell viability.

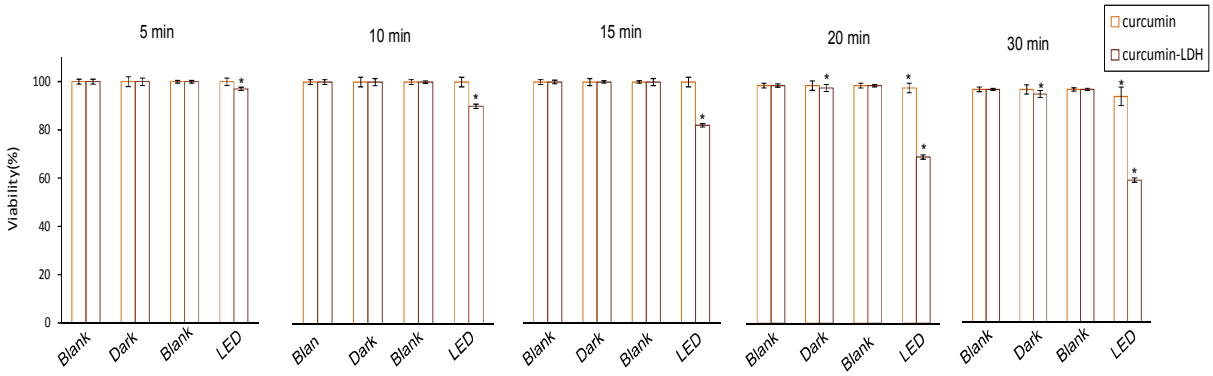


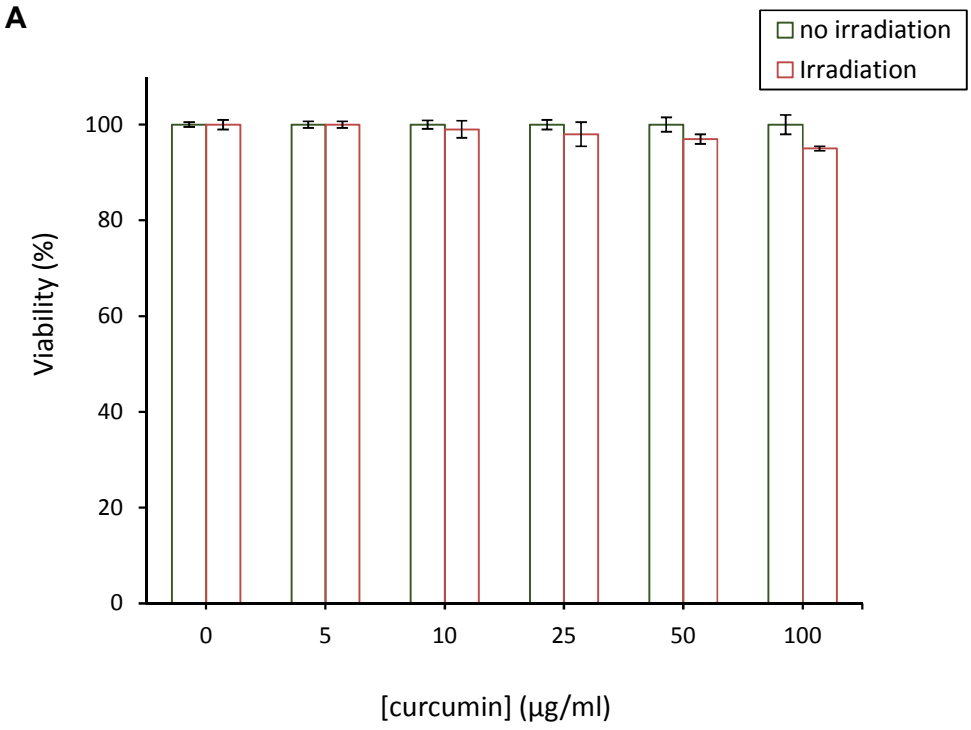
Figure 5. Viability of cells treated with 25 μg/ml of free curcumin and equivalent concentration of curcumin–LDH complex for 24 h and then one group kept in dark and another exposed to blue LED for 5,10,15,20 and 30 min, respectively. The results are expressed as the mean ± SD (n = 3, *P < 0.05 compared with control (blank) group).

In vitro photodynamic activities of curcumin-LDH nanohybrid

The PDT action of curcumin-LDH photosensitizers was further studied by in vitro tests performed with MDA-MB-231 cells. The impact of curcumin-LDH concentration on PDT effectiveness was studied. The MDA-MB-231 cells were incubated in the presence of different concentrations of curcumin-LDH for 24 h, followed by washing with PBS and irradiated with blue LED (465 nm with power density: 34 mW/cm^2).

The best PDT behavior was demonstrated with the dosage of 100 μg/ml of curcumin- LDH nanohybrid (the difference between the dotted green and dashed red bar: 38) (figure 6 B). For comparison, the PDT performance of free curcumin was also studied by incubating MDA-MB-231 cells with medium containing free curcumin.

After 24 h incubation of cells with 100µg/ml of free curcumin the cell viability was 99% (no irradiation) and 95% (irradiation), indicating some cytotoxicity as well as rather poor PDT effectiveness (figure 6 A). In the case of curcumin-LDH nano hybrid (Figure 6 B), the cell viability after 24 h of treatment was found to be 90% (no irradiation) and 52% (irradiation) respectively, which demonstrates largely-enhanced PDT effectiveness and acceptable cytotoxicity.



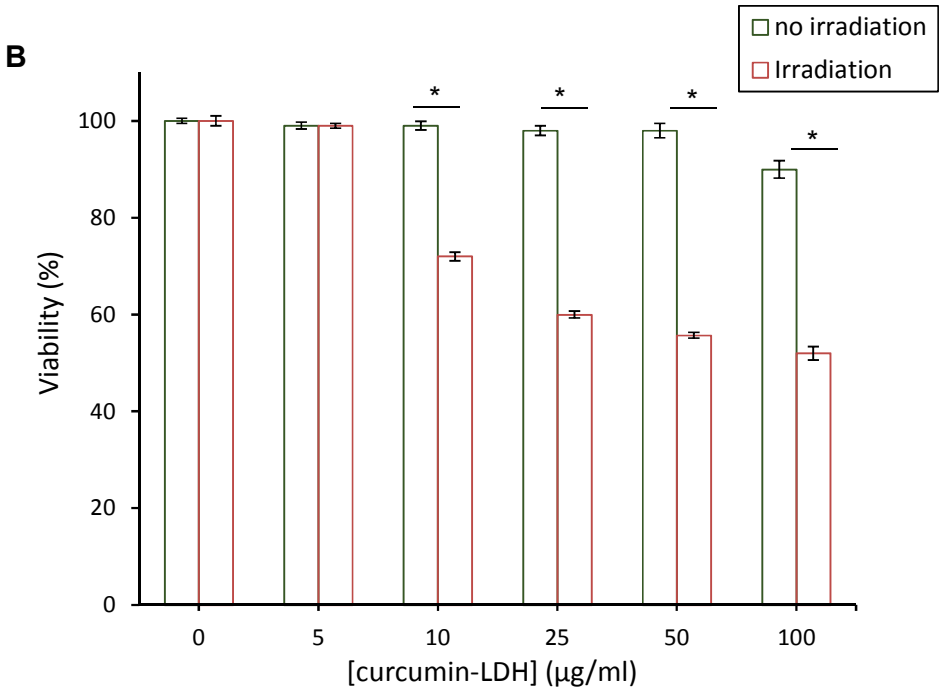


Figure 6. The PDT performance of (A) curcumin and (B) curcumin-LDH with various concentrations after 24 h incubation. The MDA-MB-231 breast cancer cells were used in these cases. The results are expressed as the mean ± SD (n = 3, *P < 0.05 compared with no irradiation group).

Microscopic study

In order to visualize the phototoxicity effect, images of MDA-MB-231 cells treated with curcumin and curcumin-LDH nanohybrid, without irradiation and under irradiation were studied under invert microscopy. As seen in figure 7, there is morphological difference between cell treated with free curcumin and curcumin-LDH nanohybrid (C and F panels).

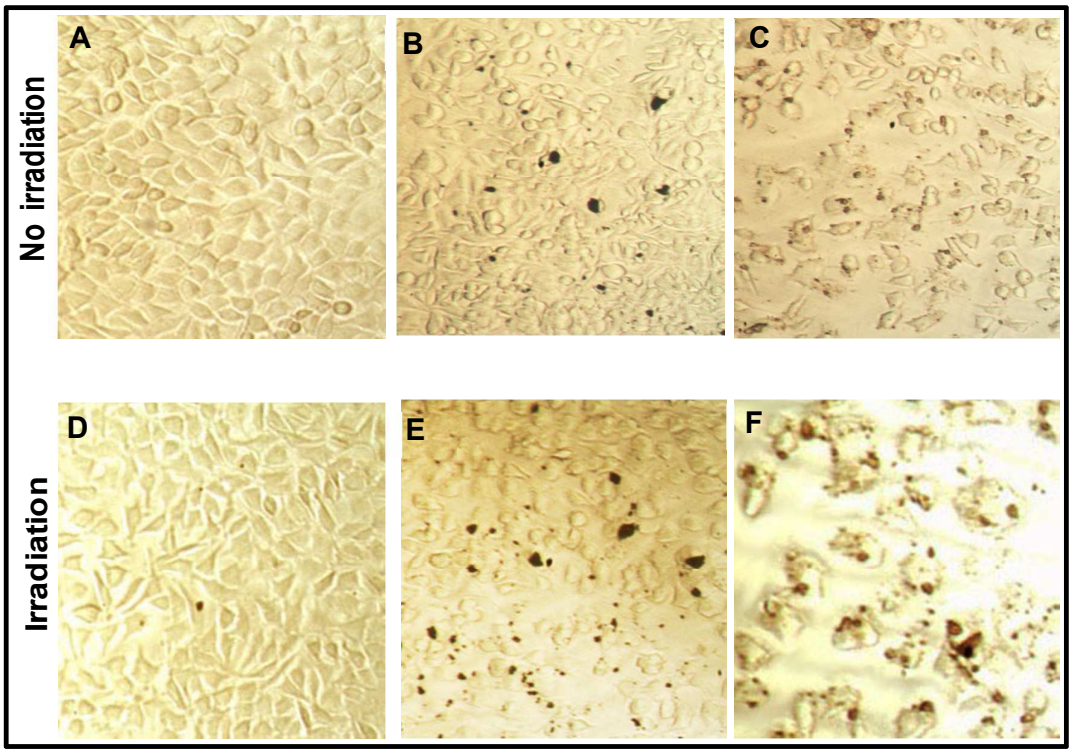


Figure 7. Invert microscopy images (40 X) of MDA-MB-231 cells treated with free curcumin and curcumin-LDH (100 µg/ml, 24 h incubation) without irradiation: (A) blank, (B) curcumin and (C) curcumin-LDH. MDA-MB-231 cells treated with curcumin and curcumin-LDH (100 µg/ml, 24 h incubation) under irradiation (30 min irradiation): (D) blank, (E) curcumin and (F) curcumin-LDH.

Discussion

From the past till today, cancer therapy by natural anticancer compound with low Sid effects in comparison to chemical anticancer drugs has attracted most scientists in the world. Curcumin is the major constituent of turmeric powder, extracted from the rhizomes of the plant Curcuma longa [24]. Various researches have done on curcumin applications as a natural polyphenolic compound for its anticancer effect against many different types of cancers [25,26]. However, the poor solubility of curcumin leads to low bioavailability and limit the potential

effect of curcumin in cancer therapy. Recent studies have shown that toxic effect of curcumin can improve by encapsulating in liposome, micelles, silica and cyclodextrine nanoparticles [27,28]. There are few studies about potential role of curcumin as photosentitizer in photodynamic therapy[29]. Until now there is now any report on curcumin-LDH nanohybrid application in photodynamic therapy. The present study describes phototoxicity effect of curcumin-LDH nanohybrid in comparison to free curcumin in photodynamic therapy of breast cancer cells.

From the results it is concluded that the red shift in curcumin-LDH UV-Vis spectra is related to curcumin intercalation in LDH and chemical environment around the molecule that decreases the water repulsion and chemical potential for host molecule. Because of hydrophobic structure and poor water solubility of curcumin the absorbance of free curcumin is low but when the curcumin intercalates in the LDH layers the absorbance peak increases. Increasing in absorbance peak of curcumin-LDH demonstrated that the curcumin in LDH nanohybrid well dispersed in solution and stable dispersion obtained. The consequently swelling of LDH nanoparticles makes stable dispersion for solutions [30]. These findings are consistent with the results of previous studies showing that the curcumin intercalated LDHs are more stable and also have release properties with future potential in therapeutic applications [31]. Reduction in fluorescence spectra of curcumin-LDH demonstrates that by intercalating of fluorescence dye (curcumin) in the layered structure (LDH) some of exited electrons could not return to lower electron layers therefore, electron transferring decreases by intercalating and also quenching effect of LDH on curcumin fluorescence [32]. To the best of our knowledge, oxygen molecule could work as quencher [33]. It could be suggested that by reduction in fluorescent intensity of exited curcumin by oxygen in LDH nanohybrid complex (FRET like reaction) the population of excited electron increases and subsequently it could generates more singlet oxygen by using excited electrons in radical production route [34–

[36]. It should be noted that irradiation could increases the excited electrons and singlet oxygen production in PDT, too [37]. Further experiments have been done for approving the intercalation of curcumin in the LDH layers such as XRD, SEM and TEM techniques. According to sharp and symmetric reflections in LDH XRD patterns it can be concluded that there is a well-crystalized hydrotalcite-like phase for LDH structure. The left shift in 2θ (2θ=22.46 (for LDH) and 2θ=22.26 (for Curcumin-LDH) and sharp lines at low angle clearly demonstrate the intercalation of curcumin in the interlayer region of LDH complex. The basal interlayer space (d_{003} was increased by intercalating of Curcumin in LDH layers (d_{LDH}=7.98 A˚ and $d_{Curcumin-LDH}$=8.52 A˚). The obtained results are in agreement with previous studies indicates that the curcumin can be intercalated in the interlayer region of LDH [31]. Hexagonal lamellar structural morphology and relative uniform size for synthesized LDH and nanohybrid represents in SEM and TEM images. The narrow size distribution in crystalline beads (70-90 nm) confirms that hydrophilic properties of LDH chemical structure could improve the curcumin water solubility and stability of curcumin-LDH nanohybrid dispersion.

The phototoxicity of void LDH results clearly shows that LDH has not any significant toxicity on the cell incubated with LDH kept dark or irradiated. These observations suggest that LDH can be use as biocompatible drug carrier in photosensitizer delivery to cancer cells. Recently, LDHs introduced as a pH-responsive controlled release systems [21]. As described previously, at pH 7.4, the amount of ZnPcPS4 released from LDH–ZnPcPS4 was lower than the pH 6.5 and 5.0, typical of those in tumor stroma or subcellular organelles such as cytoplasm and endosomes [38]. Therefore, the LDH nanohybrid could minimizing photosentitizer release in the bloodstream and favorably quick releasing photosentitizer from LDH once reaches the acidic tumor stroma which is certainly favorable for targeted cancer treatment[21]. Pre-incubation time study reveals that in dark, the cell viability for LDH, curcumin and curcumin-LDH nanohybrid have same pattern and does not show significant

difference. Irradiation in various times with blue LED clearly shows the phototoxicity of curcumin-LDH nanohybrid. The obtained results from photodynamic studies reveal that curcumin-LDH nanohybrid exhibit the excellent photodynamic activity in comparison with free curcumin as photosensitizer. Therefore, with no doubt, the water dispersion and solubility effect of LDH could improve the photosensitizer penetration and distribution in cells for effective photodynamic therapy performance. Moreover, the deformation and destruction of breast cancer cells due to good penetration and distribution of curcumin-LDH nanohybrid determine using invert microscopy.

Conclusion

Taking all together, our investigation suggests that curcumin–LDH nanohybrid shows more phototoxic effects on breast cancer cells as compared to free curcumin due to an increase in aqueous solubility and stability of curcumin at physiological pH. In addition, photodynamic activity of curcumin in nanohybrid is enhanced as indicated by an increase in cell killing. Increased photodynamic activity of curcumin delivered through LDH in breast cancer cells suggests that LDH could be a powerful delivery vehicle for improving photodynamic efficacy of curcumin for breast cancer treatment. Although our experiments on breast cancer cells confirms the phototoxic effect of curcumin-LDH nanohybrid but the precise mechanism is still unknown and remain to be elucidated.

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