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Original Article

Interaction of curcumin on cisplatin cytotoxicity in HeLa and HepG2 carcinoma cells

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ABSTRACT

Background and Aims: Our study aimed to evaluate how curcumin affect cisplatin cytotoxicity in human cervical carcinoma (HeLa), human hepatocellular carcinoma (HepG2), and Chinese hamster lung fibroblast (V79) cells.
Methods: The cytotoxicity was evaluated by MTT assay.
Results: The IC_{50} values of curcumin were 404 µM and 320 µM in HeLa cells; 236 µM and 98.3 µM in HepG2 cells; 877 µM and 119 µM in V79 cells; for 24 h and 48 h, respectively. The IC_{50} values of cisplatin were 22.4 µM and 12.3 µM in HeLa cells; 25.5 µM and 7.7 µM in HepG2 cells; 15.4 µM and 4.9 µM in V79 cells; for 24 h and 48 h, respectively. Curcumin significantly decreased cisplatin cytotoxicity at 500 µM in HeLa cells and above 250 µM and 125 µM in HepG2 cells, for 24 h and 48 h, respectively. In V79 cells, curcumin significantly decreased the IC_{50} values of cisplatin above 500 µM and 125 µM for 24 h and 48 h.
Conclusion: The results might contribute to the anticancer effect of the curcumin-cisplatin combination in cervical and hepatocellular carcinoma, but in order to support this result and determine its interactions with antineoplastic drugs, further studies are needed.

Keywords: Curcumin, cisplatin, cytotoxicity, HeLa cells, HepG2 cells

INTRODUCTION

Multidisciplinary treatments, including surgical treatment, radiotherapy and chemotherapy are applied to patients with cancer. Recently, new and improved therapies have been investigated to improve the survival and quality of life of patients with various types of cancer (Falzone, Salomone, & Libra, 2018). Combination of antineoplastic drugs with antioxidant agents has become a promising method for cancer treatment; however, knowing how to improve the effect of the combination therapy is of great significance. Therefore, nowadays the combination therapies have been investigated with the aim of increasing anticancer effects and decreasing cytotoxicity (Chen, Xu, & Chen, 2015; Adahoun, Al-Akhras, Jaafar, & Bououdina, 2017; Perrone, et al., 2015; Taner, et al., 2014; Yang, Shin, & Cho, 2014).

Cisplatin, one of the chemotherapeutic agents, is often used in the treatment of solid tumors such as testis, over, bladder, prostate, cervix, and lung cancer (Rosenberg, 1985). It has severely dose-limiting toxicity including ototoxicity, neurotoxicity, nephrotoxicity and cardiotoxicity. One of the mechanisms responsible for the adverse effects of cisplatin is the stimulation of oxidative stress (Florea, & Busselberg, 2011; Dugbartey, Peppone, & de Graaf, 2016). Many researchers have shown that it is useful to combine cisplatin with antioxidant agents in order to increase the effectiveness of cancer chemotherapy, decrease resistance development and reduce cytotoxicity. However, supporting studies on this issue are still insufficient. (Florea, & Busselberg, 2011).

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Curcumin, (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), is a yellow-orange polyphenol derived from the plant *Curcuma longa L. (C. longa)*, which is commonly called "turmeric, saffron root and jellyfish". Extensive research has indicated that curcumin offer a lot of benefits including antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic, antidiabetic, antibacterial, and antiviral properties (Menon & Sudheer, 2007; Koohpar, Entezari, Movafagh & Hashemi, 2015; Bulboacă, et al. 2009; Çıkrıkçı, Mezioğlu, Yılmaz, 2008). Nowadays, it is consumed as a food supplement in many countries owing to its strong antioxidant activity (Kunnumakkara, Bordoloi, Padmavathi, Monisha, Roy, Prasad, & Aggarwal, 2017b; Guzman, 2019). Despite its reported benefits via inflammatory and antioxidant mechanisms, one of the major problems with ingesting curcumin by itself is its poor bioavailability, which appears to be primarily due to poor absorption, rapid metabolism, and rapid elimination. Curcuminoids have been approved by the Unites state Food and Drug Administration (FDA) as "Generally Recognized As Safe" (GRAS), and good tolerability and safety profiles have been reported even at doses between 4 and 8 g/day (Hewlings, & Kalman, 2017; Gupta, Patchva, & Aggarwal, 2013; Anand, Kunnumakkara, Newman, & Aggarwal, 2007). Studies conducted in both animals and humans have suggested that this polyphenol is effective and safe for the prevention and treatment of many diseases. Curcumin-induced sensitization to cisplatin have been observed in many cancer cell models (Florea, & Busselberg, 2011; Guzman, 2019) such as breast cancer (Zou et al., 2018), cervical cancer (Roy & Mukherjee, 2014), bladder cancer (Zhang, Yong, Wu & Liu., 2014), human neuroblastoma cells (Sukumari-Ramesh et al., 2011), laryngeal carcinoma (Zhang, Tianyu, Lianji, Hui, Dan & Chunshun, 2013), human colorectal cancer (Wang, Liu & Su, 2014) and lymphoma cells (Zhang et al., 2014).

Curcumin has been suggested to exhibit chemopreventive and therapeutic effects through multiple mechanisms, as shown by several preclinical and clinical studies (Aggarwal, Prasad, Sung, Krishnan, & Guha, 2013; Shehzad, Lee, & Lee, 2013; Kunnumakkara, Bordoloi, Harsha, Banik, Gupta, & Aggarwal, 2017a). However, whether or not the use of curcumin during chemotherapy cause interactions with efficacy of antineoplastic drugs is not well known. The underlying cellular and molecular processes are not clear, particularly on cancer; thus, new and advanced studies are needed to clarify the effects of curcumin in cancer cells (Çıkrıkçı, Mozioğlu, Yılmaz, 2008; Nagpal, & Sood, 2013; Bansal, Goel, Aqil, Vadhanam, & Gupta, 2011). More in vitro studies with other cancer cells as well as in vivo studies are suggested at different doses.

In this study, the cytotoxic effects of anticancer drug cisplatin were investigated in combination with curcumin which is known to be significant biological effects and of which the importance has been increasingly in recent studies, in different cell lines including human cervical carcinoma (HeLa) cells, human hepatocellular carcinoma (HepG2) cells, and Chinese hamster lung noncancer fibroblast (V79) cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HeLa and HepG2 carcinoma cells were selected in our study, since cisplatin is mainly used for the treatment of malignant tumors including cervical and liver carcinomas. V79 cells, non-tumor cell lines, has been widely used to study the toxicity of a wide variety of cytotoxic agents. It has been preferred as a healthy cell line in terms of revealing its difference with non-cancerous cells compared to cancerous cells including HeLa and HepG2 cells.

MATERIALS AND METHODS

Chemicals

The chemicals used in the experiments were purchased from the following suppliers: cisplatin (Koçak Farma, Turkey); curcumin, dimethyl sulfoxide (DMSO), Dublecco's modified Eagle's medium (DMEM), ethanol, fetal bovine serum (FBS), MTT, penicillin-streptomycin, trypan blue, trypsin–EDTA, RPMI 1640 medium, Dulbecco's phosphate buffered saline (PBS) from Sigma (St. Louis, MO, USA); millipore filters from Millipore (Billerica, MA, USA), all other plastic materials from Cornings (Corning Inc., NY, USA). The purity of curcumin is ≥80%.

Cell culture

HeLa, HepG2 and V79 cells were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). V79 cells were grown in RPMI-1640 medium with 10% heat-inactivated FBS, and 1% penicillin-streptomycin solution (10000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl), and 2mM L-glutamine at 37°C in a humidified atmosphere of 5% CO_2. HeLa and HepG2 cells were cultured in in Dulbecco's Modified Eagle Medium (DMEM) containing low glucose (1 g/L) and sodium pyruvate and supplemented with 10% heat-inactivated fatal bovine serum (FBS), 2mM L-glutamine and 1% penicillin-streptomycin solution (10000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. The cells were subcultured in 75 cm^2 cell culture flasks. The medium was changed every 3 days. The passage numbers used in our study for all cell lines were between passage 10 and passage 12.

Determination of cytotoxicity

The cytotoxicity of curcumin and cisplatin were measured in HeLa, HepG2, and V79 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase (Mosmann, 1983; Hansen, & Nielsen, 1989).

After growing for 2 weeks, the cells were plated at 1×10^4 cells/well by adding 200 µL of a 5×10^4 cells/mL suspension to each well of a 96-well tissue culture plate and allowed to grow for 24 h before treatment. The number of cells was calculated by trypan blue dye exclusion.

The stock solution of curcumin was freshly prepared in DMSO as solvent control and filtered with millipore filters (0.20 µm). DMSO concentration was not exceed 0.5% (v/v) in medium. The cells were treated alone with CUR in a wide range of concentrations (1.95-2000 µM) or cisplatin (0.49-500 µM) in the

related culture medium for 24 h and 48 h. Negative control experiments were carried out with the culture medium containing DMSO (0.5%) or PBS (1%), for curcumin and cisplatin, respectively. After the values of IC_{50} were determined, the effects of curcumin in a wide range of doses on the cytotoxicity of cisplatin were evaluated in all cells for 24 h and 48 h.

There is no reference value for the selected doses. The IC_{50} values determined in our experiment were taken into account in the dose selection, since cytotoxic doses vary greatly depending on the cell type and number of passages studied, the exposure time and the cytotoxicity method used in the literature. After the effects of curcumin on cell viability in the wide dose range were determined for one non-cancer cell and two different cancer cells at two different periods of 24 h and 48 h, the result was extended by studying the wide dose range including the cytotoxic dose. IC_{50} value, kills 50% of the living cell population, is a dose which shows cytotoxicity of substances. The dose of cisplatin, which is cytotoxic, is also the target dose in chemotherapy, because the aim is to prevent cancer cell proliferation. In order to show how curcumin taken in various forms, such as food supplements or nano-drug formulations, changes the cytotoxic effect of cisplatin, the combination of curcumin in a wide dose range with the IC_{50} value of cisplatin was preferred. The results are crucial in terms of revealing the synergistic or antagonistic effects in terms of cell viability between cisplatin and curcumin, and these results also contribute to predicting possible interactions. At the end of the treatment (24 h and 48 h), the cells were then incubated with 0.5 mg/mL MTT solution for another 4 h at 37°C. Then the medium was discarded and washed with PBS. DMSO (100 µl) was added and the plates were shaken for 5 min gently. The formazan crystals were dissolved in DMSO and absorbance of each sample was measured at 570 nm using the microplate reader (SpectraMax M2, Molecular Devices Limited, Berkshire, UK). Results were expressed as the mean percentage of viable cells compared with non-treated controls.

The percentage of cell viability was calculated using the formula:

"Percentage of cell viability = (The absorbance of sample/ control) x 100"

The cytotoxic concentration that killed cells by 50% (IC_{50}) was determined from absorbance versus concentration curve.

Statistical analysis

All experiments were carried out in quadruplicate. The results were given as the mean ± standard deviation. The statistical analysis was performed with software programs *"SPSS 10.5"* (Statistical Package for the Social Sciences, Chicago, IL, USA). The distribution of the data was checked for normality using the Kolmorog-Smirnov test. The means of data were compared by the one-way variance analysis test and post hoc analysis of group differences was performed by least significant difference (LSD) test. *P* value of less than 0.05 was considered as statistically significant.

RESULTS

Effects of curcumin and cisplatin on the viabilities of HeLa, HepG2, and V79 cells

In HeLa cells, the results of curcumin cytotoxicity are given in Figure 1. Curcumin did not cause significant cytotoxic effect at the concentrations of 1.95-250 µM and at the concentrations of 1.95-125 µM when compared to the negative control for 24 h and 48 h incubation, respectively; however, the cell viabilities were significantly decreased above 500 µM and 250 µM concentrations of curcumin ($p<0.05$) for 24 h and 48 h incubation, respectively, in a dose-dependent manner. The IC_{50} value of curcumin 403.5 µM and 320.2 µM for 24 h and 48 h, respectively (Figure 1).

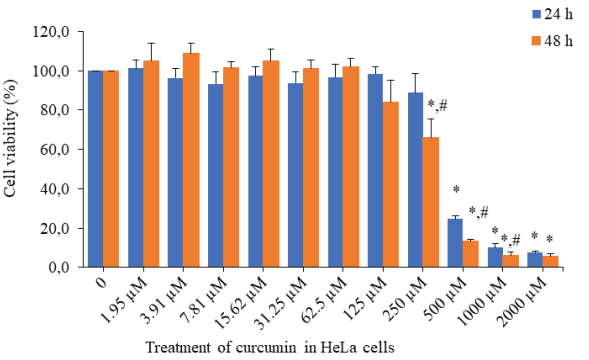

Figure 1. Cytotoxic effects of curcumin on HeLa cells viability for 24 h and 48 h. The values were given as the mean ± standard deviation (n=4). *$p<0.05$, compared to negative control (untreated cells) (0.5% DMSO). #$p<0.05$, 24-hour cell viability was compared to 48-hour cell viability.

The results of cisplatin cytotoxicity in HeLa cells are given in Figure 2. Cisplatin did not cause significant cytotoxic effect at the concentrations of 0.49-7.81 µM and at the concentrations of 0.49-3.91 µM when compared to the negative control for 24 h and 48 h, respectively; however, the cell viabilities were significantly decreased above 15.63 µM and 7.81 µM of cisplatin for 24 h and 48 h incubation, respectively, in a dose-dependent manner ($p<0.05$). The IC_{50} values of cisplatin were 22.4 µM and 12.3 µM for 24 h and 48 h, respectively (Figure 2).

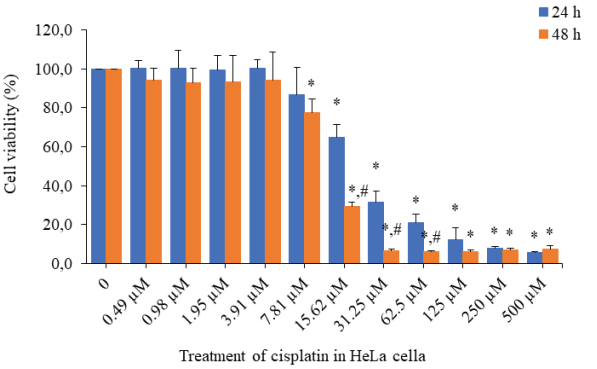

Figure 2. Cytotoxic effects of cisplatin on HeLa cells viability for 24 h and 48 h. The values were given as the mean ± standard deviation (n=4). *$p<0.05$, compared to negative control (untreated cells) (PBS). #$p<0.05$, 24-hour cell viability was compared to 48-hour cell viability.

In HepG2 cells, the results of curcumin cytotoxicity are given in Figure 3. Curcumin did not cause significant cytotoxic effect at the concentrations of 1.95-125 μM and at the concentrations of 1.95-31.25 μM when compared to the negative control for 24 h and 48 h incubation, respectively; however, the cell viabilities were significantly decreased above 250 μM and 62.5 μM concentrations of curcumin (p<0.05) for 24 h and 48 h incubation, respectively, in a dose-dependent manner. The IC_{50} value of curcumin were 235.8 μM and 98.3 μM for 24 h and 48 h, respectively (Figure 3).

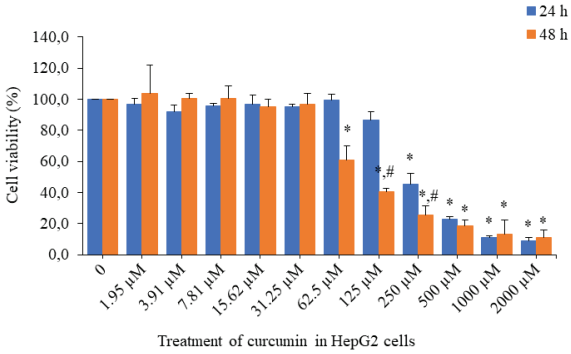

Figure 3. Cytotoxic effects of curcumin on HepG2 cells viability for 24 h and 48 h. The values were given as the mean ± standard deviation (n=4). *p<0.05, compared to negative control (untreated cells) (0.5% DMSO). #p<0.05, 24-hour cell viability was compared to 48-hour cell viability.

The results of cisplatin cytotoxicity in HepG2 cells are given in Figure 4. Cisplatin did not cause significant cytotoxic effect at the concentrations of 0.49-7.81 μM and at the concentrations of 0.49-3.91 μM when compared to the negative control for 24 h and 48 h, respectively; however, the cell viabilities were significantly decreased above 15.63 μM and 7.81 μM of cisplatin for 24 h and 48 h incubation, respectively, in a dose-dependent manner (p<0.05). The IC_{50} values of cisplatin were 25.5 μM and 7.7 μM for 24 h and 48 h, respectively (Figure 4).

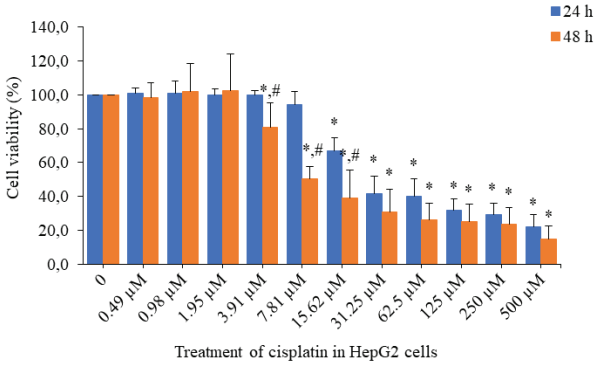

Figure 4. Cytotoxic effects of cisplatin on HepG2 cells viability for 24 h and 48 h. The values were given as the mean ± standard deviation (n=4). *p<0.05, compared to negative control (untreated cells) (PBS). #p<0.05, 24-hour cell viability was compared to 48-hour cell viability.

In V79 cells, the results of curcumin cytotoxicity are given in Figure 5. Curcumin did not cause significant cytotoxic effect at the concentrations of 1.95-250 μM and at the concentrations of 1.95-62.5 μM when compared to the negative control for 24 h and 48 h incubation, respectively; however, the cell viabilities were significantly decreased above 500 μM and 125 μM concentrations of curcumin (p<0.05) for 24 h and 48 h incubation, respectively, in a dose-dependent manner. The IC_{50} value of curcumin were 876.7 μM and 118.7 μM for 24 h and 48 h, respectively (Figure 5).

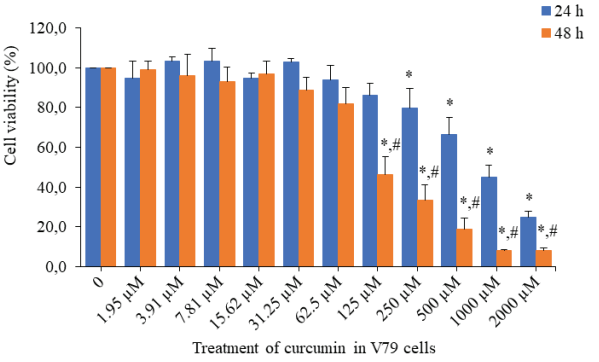

Figure 5. Cytotoxic effects of curcumin on V79 cells viability for 24 h and 48 h. The values were given as the mean ± standard deviation (n=4). *p<0.05, compared to negative control (untreated cells) (0.5% DMSO). #p<0.05, 24-hour cell viability was compared to 48-hour cell viability.

The results of cisplatin cytotoxicity in V79 cells are given in Figure 6. Cisplatin did not cause significant cytotoxic effect at the concentrations of 0.49-7.81 μM and at the concentrations of 0.49-1.95 μM when compared to the negative control for 24 h and 48 h, respectively; however, the cell viabilities were significantly decreased above 15.63 μM and 3.91 μM of cisplatin for 24 h and 48 h incubation, respectively, in a dose-dependent manner (p<0.05). The IC_{50} values of cisplatin were 15.4 μM and 4.9 μM for 24 h and 48 h, respectively (Figure 6).

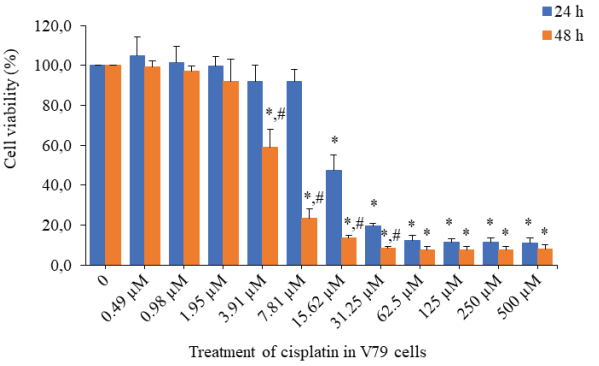

Figure 6. Cytotoxic effects of cisplatin on V79 cells viability for 24 h and 48 h. The values were given as the mean ± standard deviation (n=4). *p<0.05, compared to negative control (untreated cells) (PBS). #p<0.05, 24-hour cell viability was compared to 48-hour cell viability.

Effects of curcumin on cisplatin cytotoxicity in HeLa, HepG2, and V79 cells

In HeLa cells, the effects of curcumin at the concentrations of 15.6-500 μM on cisplatin cytotoxicity are shown in Figure 7, for 24 h and 48 h incubation. As shown in Figure 7a, curcumin

did not change the IC_{50} value of cisplatin (20 µM, approximately) at the concentrations of 15.6-250 µM for 24 h incubation; however, the IC_{50} value of cisplatin was significantly reduced at the concentration of 500 µM of curcumin (1.83 fold, vs. IC_{50} of cisplatin) when compared to the negative control for 24 h incubation ($p<0.05$). As shown in Figure 7b, when compared to the negative control, curcumin did not change the IC_{50} value of cisplatin (10 µM, approximately) at the concentrations of 15.6-250 µM for 48 h incubation; however, the IC_{50} value of cisplatin was significantly reduced at concentrations of 500 µM of curcumin (1.41 fold for 500 µM, vs. IC_{50} of cisplatin) ($p<0.05$). As a result, curcumin reduced the cytotoxicity of cisplatin at highest doses (500 µM).

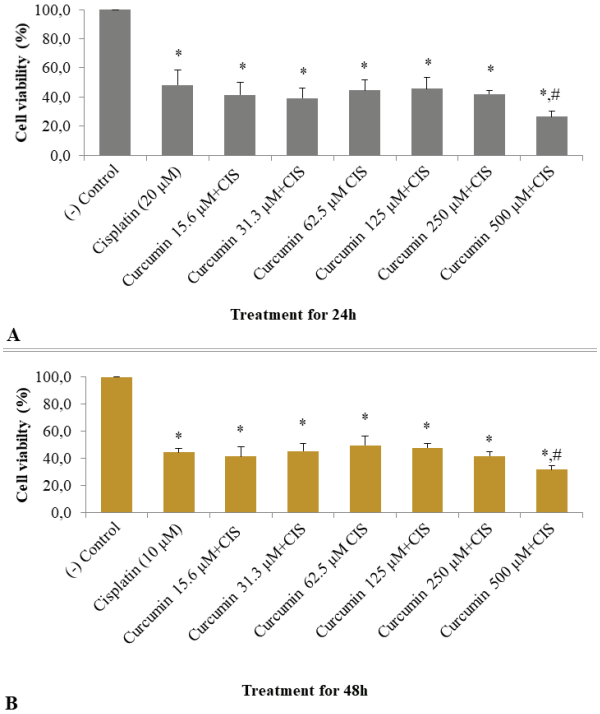

Figure 7. Effects of curcumin on the cisplatin cytotoxicity in HeLa cells for 24 h (A) and 48 h (B). The values were given as mean ± standard deviation (n=4). *$p<0.05$, compared to negative control (untreated cells) (0.5% DMSO); #$p<0.05$, compared to cisplatin as positive control (20 µM for 24 h treatment and 10 µM for 48 h treatment). CIS, cisplatin.

In HepG2, the effects of curcumin at the concentrations of 15.6-500 µM on cisplatin cytotoxicity cells are shown in Figure 6, for 24 h and 48 h incubation. As shown in Figure 8a, curcumin did not change the IC_{50} value of cisplatin (25 µM, approximately) at the concentrations of 15.6-125 µM for 24 h incubation; however, the IC_{50} value of cisplatin was significantly reduced at concentrations of 250 and 500 µM of curcumin (1.81 and 3.48 fold, vs. IC_{50} of cisplatin) when compared to the negative control for 24 h incubation ($p<0.05$). As shown in Figure 8b, when compared to the negative control, curcumin did not change the IC_{50} value of cisplatin (10 µM, approximately) at the concentrations of 15.6-62.5 µM for 48 h incubation; however, the IC_{50} value of cisplatin was significantly reduced at concentrations of 125, 250 and 500 µM of curcumin (1.71, 4.09 and 19.04 fold vs. IC_{50} of cisplatin) ($p<0.05$). As a result, curcumin reduced the cytotoxicity of cisplatin at the high doses (125-500 µM).

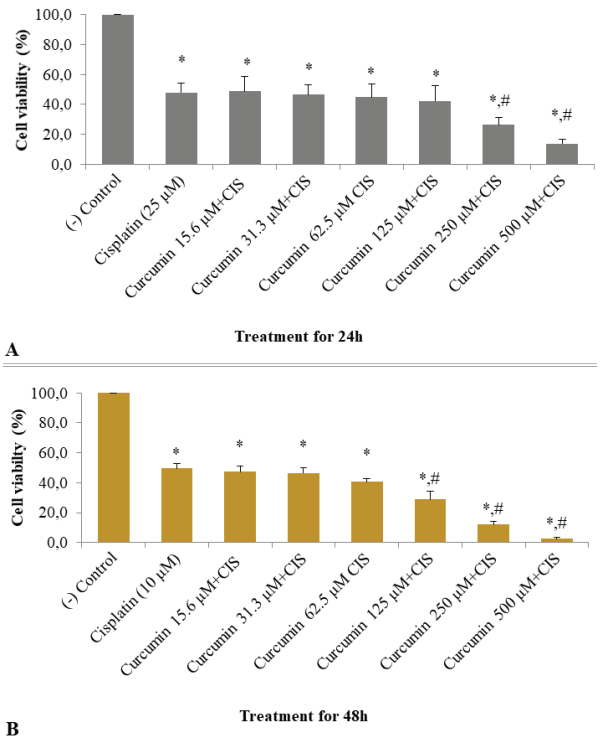

Figure 8. Effects of curcumin on the cisplatin cytotoxicity in HepG2 cells for 24 h (A) and 48 h (B). The values were given as mean ± standard deviation (n=4). *$p<0.05$, compared to negative control (untreated cells) (0.5% DMSO); #$p<0.05$, compared to cisplatin as positive control (25 µM for 24 h treatment and 10 µM for 48 h treatment). CIS, cisplatin.

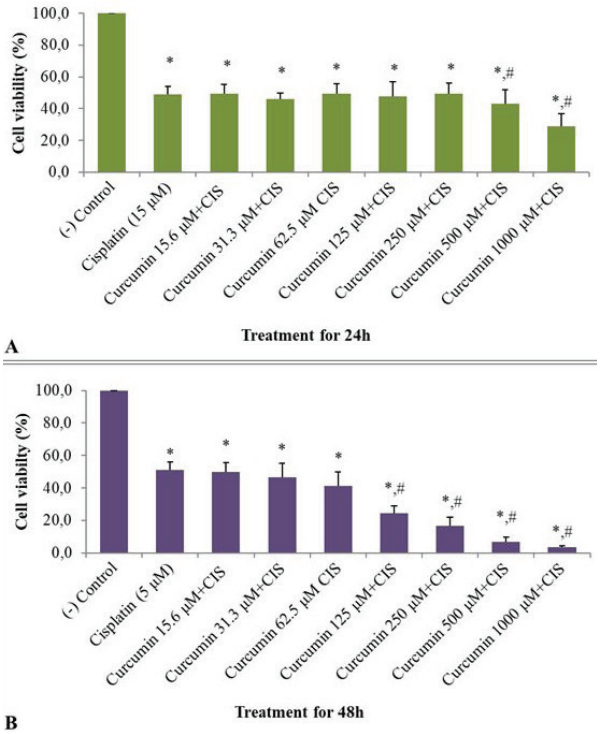

Figure 9. Effects of curcumin on the cisplatin cytotoxicity in V79 cells for 24 h (A) and 48 h (B). The values were given as mean ± standard deviation (n=4). *$p<0.05$, compared to negative control (untreated cells) (0.5% DMSO); #$p<0.05$, compared to cisplatin as positive control (15 µM for 24 h treatment and 5 µM for 48 h treatment). CIS, cisplatin.

In V79 cells, the effects of curcumin in a wide range of concentrations (15.6-1000 µM) on cisplatin cytotoxicity are shown in Figure 9, for 24 h and 48 h incubation. As shown in Figure 9a, curcumin did not change the IC_{50} value of *cisplatin* (15 µM, approximately) at the concentrations of 15.6-500 µM for 24 h incubation; however, the IC_{50} value of *cisplatin* was significantly reduced at concentrations of 1000 µM of curcumin (1.71 fold, vs. IC_{50} of cisplatin) when compared to the negative control for 24 h incubation ($p<0.05$). As shown in Figure 9b, when compared to the negative control, curcumin did not change the IC_{50} value of *cisplatin* (5 µM, approximately) at the concentrations of 15.6-62.5 µM for 48 h incubation; however, the IC_{50} value of cisplatin was significantly reduced at concentrations of 125, 250, 500 and 1000 µM of curcumin (2.08, 3.07, 7.66 and 14.76 fold vs. IC_{50} of cisplatin) ($p<0.05$). As a result, curcumin reduced the cytotoxicity of cisplatin at high doses (125-1000 µM).

DISCUSSION

Multidisciplinary treatments, including surgical treatment, radiotherapy and chemotherapy are applied for patients with cancer. Recently, new and improved therapies have been investigated to improve the survival and quality of life of cancer patients with various types of cancer (Falzone, Salomone, & Libra, 2018). Combination of antioxidant agents and antineoplastic drugs has become promising method for cancer therapy; however, knowing how to improve the effect of the combination therapy is of great importance. Therefore, nowadays the combination therapies have been investigated with the aim of increasing anticancer effect and decreasing cytotoxicity (Perrone, et al., 2015; Guzman, 2019).

As is well known, cisplatin is often used in the treatment of many types of cancer (Rosenberg, 1985). It has severely dose-limiting toxicity including ototoxicity, neurotoxicity, nephrotoxicity and cardiotoxicity. One of the mechanisms responsible for the adverse effects of cisplatin is the stimulation of oxidative stress (Florea, & Busselberg, 2011; Dugbartey, Peppone, & de Graaf, 2016). Many researchers have shown that it is useful to combine cisplatin with antioxidant agents in order to increase the effectiveness of cancer chemotherapy, decrease resistance development, and also reduce cytotoxicity. However, studies are insufficient to support this (Florea, & Busselberg, 2011).

Curcumin is considered as a supportive alternative in the treatment in terms of safe, effective and low cost in the prevention and treatment of many diseases due to primarily its antioxidant and anti-inflammatory properties. However, whether the use of curcumin during chemotherapy cause interactions with the efficacy of antineoplastic drugs is not well known. The cellular and molecular processes should be clarified in cancer cells, with advanced studies (Aggarwal, Kumar, & Bharti, 2003; Moron, Montano, Salvador, Robles, & Lazaro, 2010).

Present study aimed to explains the potential synergistic cytotoxic activity of the well-known herbal origin secondary metabolite, curcumin and the clinically used drug, cisplatin. We investigated the cytotoxicity of HeLa, HepG2 and V79 cells treated with curcumin and cisplatin alone or in combination for 24 h and 48 h using MTT, a colorimetric analysis that evaluates cell metabolic activity. It seems that the cytotoxicity profiles of curcumin and cisplatin alone were different depending on the dose and time. The effects of curcumin on cell viability measured after 24 h and 48 h incubation in HeLa, HepG2, and V79 cells in a wide range of doses (1.95-2000 µM) were evaluated. In our study, IC_{50} values of curcumin were determined as 404 µM, 236 µM, and 877 µM in HeLa, HepG2 and V79 cells, respectively, for 24 h incubation. Curcumin cytotoxicity in HepG2 cells was ~ 1.7 times higher compared to HeLa cell and ~ 3.7 times higher compared to V79 cell, for 24 h. For 48 h incubation, IC_{50} values of curcumin were determined as 320 µM, 98.3 µM, and 119 µM in HeLa, HepG2 and V79 cells, respectively. Curcumin cytotoxicity in HepG2 cells was ~ 3.2 times higher compared to HeLa cell and ~ 1.2 times higher compared to V79 cell, for 48 h. When 48 h incubation is compared to 24 h incubation, the cytotoxicity of curcumin was determined as ~ 1.3 times higher in Hela cells, ~ 2.4 times higher in the HepG2 cells and ~ 7.4 times higher in the V79 cells. The effects of cisplatin on cell viability measured after 24 h and 48 h in V79, HeLa, and HepG2 cells were evaluated at the concentrations of 0.49-500 µM. IC_{50} values of cisplatin were determined as 22.4 µM, 25.5 µM, and 15.4 µM at 24 h exposure and 12.3 µM, 7.7 µM, and 4.9 µM at 48 h exposure, for HeLa, HepG2 and V79 cells, respectively. When 48 h incubation is compared to 24 h incubation, the cytotoxicity of cisplatin was determined as ~ 1.8 times higher for HeLa cells, ~ 3.3 times higher for the HepG2 cells and ~ 3.1 times higher for the V79 cells. In our study, it was determined whether or not the combination of curcumin with cisplatin increased cytotoxicity when compared to positive control (cisplatin treatment at IC50 doses) in the selected cancer (HeLa and HepG2) and normal (V79) cell lines. In HeLa cells, curcumin significantly reduced the IC_{50} value of cisplatin (20 µM (24 h) and 10 µM (48 h), approximately) at the concentration of 500 µM for 24 h, at the concentrations of 250-500 µM for 48 h incubation, respectively. In HepG2 cells, curcumin significantly reduced the IC_{50} value of cisplatin (25 µM (24 h) and 10 µM (48 h), approximately) at the concentrations of 250-500 µM for 24 h, at the concentration of 125-500 µM for 48 h incubation, respectively. In V79 cells, curcumin significantly reduced the IC_{50} value of cisplatin (15 µM (24 h) and 5 µM (48 h), approximately) at the concentration of 500 µM for 24 h, at the concentrations of 125 µM and 500 µM for 48 h incubation, respectively.

As shown in our findings, the combination of cisplatin with curcumin inhibited cell viability and significantly showed an agonist effect in selected cancer cells in time and dose dependent manner. Our results suggest that a curcumin-cisplatin combination could be useful as a therapeutic agent for human cervical and hepatocellular carcinoma and add a new perspective to anticancer treatment.

Consistent with our findings, it has been reported that curcumin could inhibit cell proliferation in the various cancer cell lines in many studies. There are many studies in which the possible mechanism underlying the cytotoxic effect of curcumin is associated with apoptosis. In a study investigating the effect and possible mechanism of curcumin, cisplatin and their synergistic effect, both curcumin and cisplatin have been shown to enhance the growth inhibition and apoptosis of human lung adenocarcinoma A549 cells in a concentration-depen-

dent pattern, using MTT (Cao, Diao, & Xia, 2008). In another study, it was investigated the role of curcumin in lung cancer (A549) cell proliferation and apoptosis in regulating the signaling pathway which plays an important role in tumor occurrence. CCK-8 assay and EdU staining for cell proliferation and Real-time quantitative PCR for mRNA expressions and Western blot for protein levels were carry out. It was reported that curcumin (10 μM) treatment markedly inhibited A549 cell proliferation and induced apoptosis in a dose dependent manner. It was also reported that curcumin could inhibit lung cancer cell proliferation and promote apoptosis by downregulating DJ-1 to regulate the activity of PTEN/PI3K/AKT pathway (Li, Qin, & Li, 2019). It is thought that new and advanced studies should be carried in order to illuminate the underlying mechanisms of these effects of curcumin in a better way.

Some research has reported IC_{50} values of curcumin in different cells using different methods. In a study, in human breast cancer MCF-7 cells, using MTT assay, IC_{50} values of curcumin were determined as 79.58 μg/ml (~216 μM) and 53.18 μg/ml (~144 μM) and 30.78 μg/ml (~83.5 μM) in 24, 48 h and 72 h incubation, respectively (Koohpar, Entezari, Movafagh, & Hashemi, 2015). In another study, in human hepatocellular carcinoma HepG2 cells, using the neutral red uptake (NRU) assay, IC_{50} value of curcumin was reported as 41.5 μg/ml (~ 113 μM) in 72 h incubation (Abdel-Lateef et al., 2016). In another also study, IC_{50} value of curcumin were reported as 9.40 μg/ml (~25.5 μM) and 17.67 μg/ml (~47.9 μM), and 22.88 μg/ml (~62.1 μM), in MCF-7, HeLa, and HepG2 cell, respectively (Ding, Ma, Lou, Sun, & Ji, 2015). The IC_{50} values of CIS was reported to be 54.07 μM and 96.38 μM in cervical cancer cells (HeLa and Caco-2), respectively; 97.20 μM and 85.66 μM in pancreatic cancer cells (MIA PaCa-2 and BxPC-3), respectively; 14.87 μM and 77.89 μM in hepatocellular carcinoma cells (Hep-G2 and SK-HEP-1), respectively, for 24 h incubation, using MTT method (Nurcahyanti, & Wink, 2016).

Baharuddin et al., (2016) have reported that curcumin increased cisplatin-induced apoptosis and metastasis inhibition in non-small lung cancer A549 and H2170 cells. In this study, it has been concluded that curcumin (10-40 μM) is able to increase the efficacy of low dose cisplatin (3 μM) in both cells. Nowadays the combination therapies with the aim of increasing anticancer effects and decreasing toxicity have been focus of great interest. Many researchers have revealed that cisplatin has positive effects in combination with antioxidants (Mosalam, Zidan, Mehanna, Mesbah, & Abo-Elmatty, 2020; Toric, Markovic, Brala, Barbaric, 2019). Curcumin has various pharmacological properties and exhibits the ability to interact with multiple molecular targets and intracellular signaling pathways. It has antiproliferative and chemopreventive efficacy through the various pathways such as NF-κB pathway (a proinflammatory transcription factor, has a role in tumorigenesis and inflammation), STAT3 pathway (a proinflammatory transcription factor that plays a major role in the pathogenesis of various cancers), PI3K/AKT/mTOR pathway, EGFR pathway (a family of receptor tyrosine kinases, is a complex signal transduction cascade that is involved in the modulation of cell proliferation, survival, adhesion, migration, and differentiation), Nrf2 pathway (a key regulator of a variety of genes that are involved in the detoxification of electrophiles and ROS and the repair or removal of some of their damage products), Wnt/β-catenin pathway (plays a pivotal role in the regulation of cell proliferation, survival, and apoptosis), EGR-1 pathway (plays vital role in regulating growth, differentiation, and apoptosis in many cell types via the regulation of over 30 genes). Curcumin has a poor bioavailability which limits its therapeutic efficacy, and extensive research is needed to deal with the factors that cause poor bioavailability of curcumin. Besides, various analogs of curcumin and different formulations such as adjuvants, nanoparticles, and liposomes, must be be searched extensively in order to obtain its maximum efficacy and facilitate the successful prevention and treatment of cancer (Kunnumakkara, Bordoloi, Harsha, Banik, Gupta, & Aggarwal, 2017a).

There are some reports on the cytotoxic and the synergistic effects of cisplatin and curcumin either as solution or their nanosome/liposome forms on both HepG2 and HeLa cells. Ma et al., (2015) evaluated the efficacy of electrospun nanofibers co-loaded with cisplatin and curcumin to prevent local recurrence of cervical cancer after surgery. The combination of cisplatin with curcumin showed a synergetic effect on growth inhibition and apoptosis induction in HeLa cells and nanofiber-based local combination chemotherapy was more effective and less toxic than systemic combination chemotherapy for the prevention of U14 cervical cancer recurrence in mice, which may indicate its great clinical potential in the future. Chang et al., (2018) showed a combination strategy using co-loaded liposomes with cisplatin and curcumin to have the higher anti-tumor activity in vitro against HepG2 cells. They suggested that this combinational application might effectively deliver and release cisplatin and curcumin to HepG2 cells to overcome the unsatisfactory clinical outcome of cisplatin monotherapy.

CONCLUSION

In conclusion, our study shows that there are no negative interactions between curcumin and cisplatin in terms of cell viability and curcumin-cisplatin combination could be useful therapeutic agent for human cervical and hepatocellular carcinoma and add a new perspective to anticancer treatment. These findings suggest that curcumin may become as promising therapeutic candidate to increase the anticancer effects and to decrease toxicity of cisplatin in chemotherapy. However, due to curcumin having poor solubility and low bioavailability, new formulations such as nano-particles and nano-emulsion should be integrated into treatment. Our studies may thus be extended to reveal the effects of phenolic compounds including curcumin on cancer and combined with antineoplastic drugs in different doses.

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