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ORIGINAL ARTICLE

The Apoptotic Activity of Curcumin Against Oral Cancer Cells Without Affecting Normal Cells in Comparison to Paclitaxel Activity

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Abstract
Until now, chemotherapy, which has a series of side effects, has been the most widely employed treatment for different types of cancer. However, bioactive products have been utilized as alternative medicines for tumors due to their bioactivities with low or no side effects in normal cells. This research reported for the first time that curcumin (CUR) and paclitaxel (PTX) have significant anti-cancer activity against normal human gingival fibroblast (HGF) and tongue squamous cell carcinoma fibroblast (TSCCF) cell lines. The results showed that CUR (13.85 μg mL^{-1}) and PTX (8.17 μg mL^{-1}) significantly inhibited TSCCF cell viability, with no significant effect on normal HGF cells. SEM showed morphological changes in cells treated with CUR and PTX, especially with TSCCF cells, compared to HGF normal cells. For TSCCF, the results showed the highest necrosis was achieved with CUR (58.8%) and PTX (39%) as compared to the control (2.99%). For normal HGF cells, the highest early and late apoptosis was achieved with PTX. Further, DCFH-DA analyses showed no significant ROS stimulation in TSCCF and HGF cell lines treated with CUR and PTX. The ^{1}H NMR analysis results show the presence of methoxy and hydroxyl groups and aromatic hydrogens in the CUR structure. In conclusion, the results confirmed that CUR is more specific to the oral cancer cells but not normal cells by inducing apoptosis in a dose- and time-dependent manner, with decreased TSCCF cell viability, and the cytotoxicity of CUR and PTX is not through the ROS pathway.

Keywords Curcumin · Oral cancer · Paclitaxel · Fibroblast gingival cell

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Introduction

Cancer is among the leading causes of death worldwide. Despite significant cancer treatment advances, cancer incidence and mortality rates are still high [1]. Oral cancer is among the most aggressive cancers with a low survival rate because most patients respond poorly to chemotherapy treatment [2]. Therefore, the search for highly efficient anti-cancer drugs less toxic to normal cells has remained attractive in recent years [1]. Tongue cell carcinoma fibroblasts (TSCCFs) are the most prevalent types of neck and head tumors. Patients with this cancer have a poor prognosis and are predisposed to tumor return and metastasis, negatively impacting a patient's life [3]. Because of its highly invasive nature, squamous tongue carcinoma often leads to acute disorders of speech, deglutition, chewing, and cancer-related death [4]. Human gingival fibroblasts (HGFs) are the primary cells that inhabit the gingival connective tissue and are essential in maintaining and remodeling the gingival structure and extracellular matrix, tissue repair, and wound healing during their proliferation, migration, and adhesion. HGFs are easily isolated and cultured in a normal culture medium [5].

Paclitaxel (PTX) is a chemotherapy agent serving a key role in the treatment of various human cancers, such as breast, ovarian, lung, and others, by stimulating the polymerization of dipoles of tubulin to form microtubules and stabilizing microtubules by preventing depolymerization and inducing cell-cycle arrest or improving signaling factors in cancer treatments [6]. It has been reported that the cytotoxicity of PTX in vitro is based on its concentration [6]. However, PTX showed serious side effects, including gastrointestinal toxicity, myelosuppression, neurotoxicity, hepatotoxicity, cytotoxicity, and cardio-toxicity. Further, the anti-cancer activity of PTX is reduced by stimulation of drug resistance through the activation of nuclear factor B (NF-B) [7], high expression of multidrug resistance mutation 1 (MDR-1), modifications in the PTX binding site, or changes in the apoptosis protein functions [6]. Thus, there is a need to discover new and effective strategies to inhibit and reduce side effects caused by chemotherapy [7, 8].

Natural products play a key role in human healthcare and medicine; drugs derivative from natural resources are gradually being employed to treat many diseases (e.g., cancer). Curcumin (CUR) is a yellow material under the superfamily of polyphenols, usually employed as a spicing agent in food, and chemically known as diferuloylmethane (hydrophobic polyphenol) which is the active ingredient of turmeric isolated from *Curcuma longa* (dried rhizome of the plant). Several reports have shown that CUR showed anti-cancer, anti-inflammatory, and antioxidant activities [9].

Past research has reported that CUR could limit cell proliferation and induce apoptosis in various cancer cells, like breast cancer, head and neck squamous cell carcinoma, lung cancer, pancreatic adenocarcinoma, prostate cancer, and lung cancer. The negative regulation of several growth factors, carcinogenic molecules, protein kinases, transcription factors, and inflammatory cytokines mediate the anti-cancer activity of CUR. Therefore, the anti-cancer mechanisms of CUR include the increase of inhibitory growth factors (TP53, Rb, and p57Kip2) and the decrease of associated proliferative pathways (PI3K, NFκB, MAPK, and JAK-STAT), and apoptosis stimulation by a mitochondrial pathway involving cytochrome c release, caspase-8, caspase-3, BID cleavage, activation of pro-apoptotic (Bax), and inhibition of anti-apoptotic genes such as Bcl-XL. Further, CUR prompts cell cycle arrest at G1/S or G2/M phases by preventing cyclin D1 or cdc2/cyclin B expressions [9–11]. CUR can also increase intracellular calcium levels, which are involved in the induction of apoptosis by inducing changes in the capillary cell membrane [12]. In

addition, CUR can enhance the tumor necrosis factor (TNF)–related apoptosis inducing-ligand (TRAIL) in the cancer cells by controlling the release of apoptosis proteins such as ERK, Akt, and Mcl-1 [13]. Apoptosis (programmed cell death) is an important action for controlling homeostasis by removing unnecessary and abnormal cells and is among the most common processes used in cancer therapies [14–16]. Extrinsic (death receptor-dependent) and intrinsic (mitochondrial-mediated) pathways mediate apoptosis. The intrinsic process begins with the mitochondrial depolarization and loss of membrane safety, which is controlled by the protein Bcl-2, leading to the cytochrome c release into the cytosol, thus activating effector caspase-3, while the extrinsic pathway begins by extracellular death receptors, such as FAS, TNF-α, and TRAIL [17].

Therefore, the current study examined in vitro anti-cancer activity of CUR and PTX against HGF and TSCCF cell lines using different methods, including MTT assay, scanning electron microscopy, Annexin-V/propidium iodide (PI), and reactive oxygen species (ROS) activity to elucidate the dependent biochemical mechanisms and simplify the development of clinical anti-cancer drugs, further measuring the purity of the CUR compound using the nuclear magnetic factor (NMR).

Material and Methods

CUR, with a purity of > 98%, was purchased from Xi'an Sonwu Biotech, Co., Ltd., China, and dissolved in DMSO. PTX was acquired from Stigma, Baoji Guokang Bio-Technology Co., Ltd., China. Trypsin and Dulbecco's Modified Eagle Medium (DMEM) were acquired from Gibco, Life Technologies, and Waltham, Massachusetts, USA. Fetal bovine serum (FBS) was acquired from BioWest SAS, Nuaille, France. PSF was acquired from Sigma-Aldrich®, St. Louis, Missouri, USA.

Cell Culture and MTT Assay

HGF normal cells and TSCCF cancer cells were obtained from the IBRS (Tehran, Iran). Cells were grown and maintained in DMEM supplemented with 10% FBS and a 1% antibiotic antimycotic solution (PSF) in an incubator with CO_2 (5%) at 37 °C. After achieving an ~ 75% confluency, cells were detached employing trypsin (0.25%) and ethylenediaminetetraacetic acid (0.1%, Merck, Darmstadt, Germany) in phosphate-buffered saline (PBS) at 37 °C. Cells were re-suspended in DMEM with 10% FBS and 1% PSF, seeded into the 96-well plates with a density of 5000 cells/well, and incubated for 24 h. The cells were washed with PBS (pH 7.4) and incubated for 72 h in a new medium with different concentrations of CUR and PTX (1000, 500, 250, 125, 62.5, 31.25, 15.625, 0 μg mL^{-1}). The cell viability test was studied using the 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 0.5 mg mL^{-1} in PBS) dye reduction method. After 72 h of incubation, 20 μL of MTT was added to each well and incubated in the plate at 37 °C for 4 h. One hundred μL of DMSO was added with gentle shaking at 37℃ to dissolve the resulting formazan, and absorbance was gauged with an ELISA reader at 570 nm [18]. The findings were provided via an average of three independent experiments. Then the sample concentrations showing a 50% decrease in cell viability (IC_{50} values) were determined following the equation below.

$$\text{Cell viability (\%)} = \text{Mean OD/Control OD} \times 100\% \quad (1)$$

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to detect morphological changes in apoptotic cells. Treated cells-seeded slide cultures were fixed on day 3 employing 4% glutaraldehyde, followed by dehydration in graded ethanol series at 50, 70, 80, 90, and 100% (each step 3 min) and then dried at room temperature. The fixed cell seeded slide cultures were covered with a gold layer employing an Edwards Sputter Coater S150B (Burgess Hill, Edwards, UK) and checked with a Zeiss EVO LS-15 SEM (Zeiss, Oberkochen, Germany) with a fast voltage of 20 kV.

Flow Cytometry Analysis (Annexin V)

The percentage of viable cells, early and late apoptotic cells, and necrosis cells were analyzed using Annexin V-FITC and PI dual staining to examine the apoptosis-inducing effect of CUR and PTX. The harvested cells were washed for 5 min with 2 mL PBS at 1500 rpm to remove the culture medium and then washed twice with PBS and suspended in binding buffer. After that, add 5 μL Annexin V-FITC, 3 μL PI solution, and 1500 mL 1 × Binding Buffer to each sample and incubate at 4℃ for 15 min in the dark. Samples were read with BD FACS Calibur (BD Biosciences, San Jose, California, USA). FITC and PI colors were sensitized at 488 nm and were read at 530 nm and 575–610 nm [18]. The analyzed cells were assigned to four quadrants (Fig. 3). The quadrant in the lower left represents viable cells that did not take any dyes. The second quadrant in the lower right represents early apoptosis cells stained with Annexin V due to the high affinity for phosphatidylserine on the plasma membrane surface. The third quadrant in the top right represents late apoptosis cells stained with both dyes, while the fourth quadrant represents necrosis cells stained only with PI [19].

ROS Analyses

Intracellular ROS was measured with a fluorescent probe dichlorofluorescein-diacetate (DCFH-DA, Sigma). DCFH-DA diffuses across the cell membrane easily and is enzymatically hydrolyzed by intracellular esters to produce non-fluorescent DCFH, which fastest oxidizes to high-fluorescent DCF in the presence of intracellular ROS [20]. DCFH-DA staining is an easy and cost-effective method used for detecting ROS in cells, and it can be used to detect the generation of ROS after genetic modifications or chemotherapy [21]. Briefly, cells were treated with CUR and PTX for 72 h; the harvested cells were washed to remove the culture medium. After washing, add 1 mL PBS to the cell sediment. Add 2 μL of DCFH-DA to the suspension and incubate for 45 min at 37℃ in the dark condition. Finally, add 3 μL PI to samples when reading by flow cytometry.

NMR Analyses

Ten milligrams of the CUR was dissolved in 700 μL deuterated dimethyl sulfoxide (DMSO-d6) with 0.03% tetramethylsilane (TMS) in a 2-mL Eppendorf tube. The mixture was vortexed for 1 min and ultrasonicated for 15 min to solubilize the CUR; then, it was centrifuged at 13,000 rpm for 10 min. The supernatant (600 μL) was pipetted into a

5-mm NMR tube for analysis. The ^{1}H NMR spectra were obtained on a 500 MHz VModel: Innova 5 Console spectrometer (Oxford, United States), operating at 500 MHz and 25 °C, 64 scans, 2-s relaxation delay, and 8.49-min acquisition time. The spectral width was changed from 2 to 14 ppm. The presaturation (PRESAT) pulse sequence was used to decrease water signals.

Statistical Analyses

All cell viability percentages were articulated as mean ($n = 3$) per plate ± SD (standard deviation), and differences among treated and untreated cells were analyzed via one-way ANOVA. The significance level was set to $p < 0.05$ for the difference between the cells, and GraphPad Prism Software 5.0 (Boston, Massachusetts, USA) analyzed all statistical tests.

Results and Discussion

Cytotoxic Effect of CUR and PTX

An MTT assay assessed the cytotoxic effects of CUR and PTX compounds after 72 h of incubation, as shown in Fig. 1 and Table 1. The results show that CUR and PTX significantly inhibited TSCCF cell viability in a dose- and time-dependent manner with IC_{50} of 13.85 and 8.17 μg mL^{-1}, respectively. While the CUR showed less toxic activity against normal HGF cells (527.32 μg mL^{-1}) compared to PTX with IC_{50} of 108.97 μg mL^{-1} (Fig. 1b), suggesting that the effect of CUR is more specific to the oral cancer cells but not normal fibroblast cells.

CUR is a familiar pigment isolated from the *Curcuma longa* plant, and its highly omni-directional molecule with different anti-cancer mechanisms mediates chemotherapy or chemo-preventive effects on cancer with no or few side effects on normal cells, which may be due to the high cellular uptake of CUR in cancer cells than in normal cells or due to glutathione levels in cancer cells lower than in normal cells, which increases cancer cells sensitivity to CUR. In fact, CUR may have differential effects on normal cells. Interestingly, CUR did not affect normal hepatocytes in mice, which showed no superoxide generation and, thus, no cell death [22]. The sensitivity degree of the cell lines to PTX fluctuated. However, they reported a slight boost in cell viability detected at the highest doses of PTX (25–100 nM) in MDA-MB-231 cells compared with the 10 nM dose, and the PTX effects may have arrived at a plateau phase (10 nM of concentration). Therefore, the sensitivity order of the cell lines to PTX was MDA-MB-231, pII, and MCF-7 (from the high to the lower sensitive) [23].

Previous studies confirmed that the CUR and PTX could efficiently induce cell cycle arrest or cell death (including apoptosis and necrosis) against different types of cancer [24]. The CUR natural compounds show various effects in different cancer cell types that may be due to changes or differences in (1) the stability of the CUR in the culture medium; (2) the release of CUR under different conditions; (3) sensitivity of different cell types to CUR; (4) bioactive compounds cellular permeability; (5) contaminated with metal ions; (6) hydroxyl groups present in the molecule; and (7) in vivo bio-distribution [25]. Further, Koohpar et al. [13] have confirmed that the reduction in cell viability after being treated with CUR depends on the dose and duration of treatment. However, due to its low activity, CUR remains under study to attain relevant results for cancer therapies, for example,

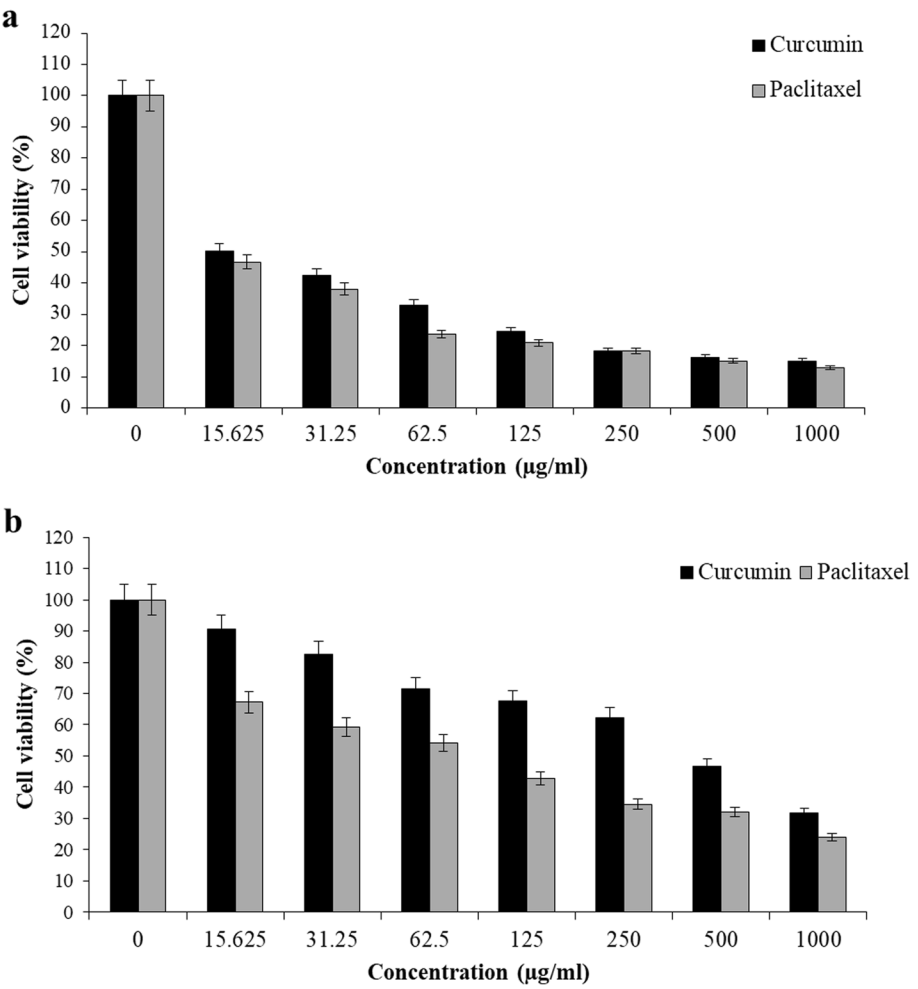

Fig. 1 Cytotoxic effects of curcumin and paclitaxel on **a** TSCCF cancer cells and **b** HGF normal cell lines

Table 1 IC_{50} value of CUR and PTX against TSCCF and HGF cell lines after 72 h treatments

Treatments/cell lines	TSCCF	HGF
Curcumin	13.85 µg mL^{-1}	527.32 µg mL^{-1}
Paclitaxel	8.17 µg mL^{-1}	108.97 µg mL^{-1}

enhancing CUR bioavailability, modifying its structure, and examining its activity against unexamined cells. Thus, CUR does not need high activity in cancer cells but must have specific targets in cancer cells, especially resistant cancer cells [26, 27].

CUR, as a potent anti-cancer factor, exhibited effects through several mechanisms such as inhibiting the NF-κβ factor, activator protein 1 (AP-1), tumor necrosis factor α (TNF-α), Lysyl oxidase (LOX), cyclooxygenase-2 (COX-2), growth factors (EGFR and HER-2), and chemokines, or by suppressing angiogenic cytokines. Further, CUR can interfere with the

cell cycle and CDK expression reduction [28]. Chakravarti et al. [29] reported that CUR could inhibit the growth of immortalized oral mucosal epithelial cells and squamous cell carcinoma cells by suppressing the phosphorylation of proteins eIF4G, 4E-BP1, eIF4B, and Mnk1with lower effects on normal oral epithelial cells (NOM9). Further, CUR could induce apoptosis in cancer cells by stimulating changes in cell membrane potential [12]. Thus, CUR showed anti-cancer effects against different types of cancer such as BT474, MBA-MB-231, SKBR3 breast cancer [12], MCF-10A (human mammary epithelial, normal), MCF-7/TH (MDR breast carcinoma) [30], head neck squamous cell carcinoma [31], and HCT116 colorectal cancer cell lines [32].

Morphological Analysis

Employing an optical and electron microscope is essential to identify morphological changes after cell death. Some researchers suggested that apoptosis can also occur during autophagy when apoptotic modulators such as Bax, Bak, or caspase are missing [33]. Morphological changes of TSCCF and HGF control cells and treated cells with CUR and PTX were assessed by SEM. Figure 2 was taken at 20 μM to check the size and morphology of the cells; the scale bar is 5 μm.

Untreated cells (control cells, Fig. 2a, d) showed well-preserved morphology (flattened as well as polygonal). The control cell was tightly adherent and enclosed with abundant microvilli, and those cells were connected firmly with neighboring cells and extended in all directions. Apoptotic body in treated TSCCF cells with CUR (Fig. 2b) was smaller in size (shrinkage) and decreased surface microvilli or completely disappeared, presenting

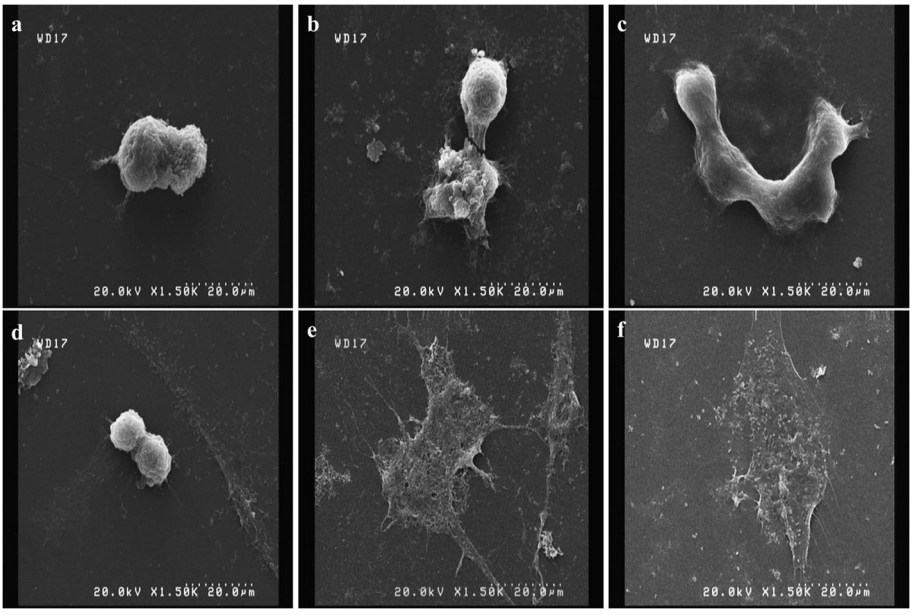

Fig. 2 Scanning electron microscope of TSCCF cancer cells (**a**, **b**, **c**) and HGF normal cells (**d**, **e**, **f**) after 72 h treatment at the IC_{50} levels for control cells, cells treated with CUR, and cells treated with PTX, respectively

membrane blebbing and loss of intercellular connections, with gradual cytoplasmic content liberation as a common morphological alteration [33, 34], while cells treated with PTX (positive control, Fig. 2c) showed morphological changes similar to those observed in cells treated with CUR. Normal cells treated with CUR showed slight morphological alterations compared to cells treated with PTX and control (Fig. 2e, f).

Flow Cytometric Analyses

Annexin V was used to determine the percentage of the apoptotic cell populations. For TSCCF (Fig. 3A), the results indicated that the percentage of viable cells decreased from 73.7% (control) to 35.7% after being treated with the CUR, followed by PTX (46.9%), while the percentage of late and early apoptosis was 4.76, 8.74%, and 1.17, 4.32% for PTX and CUR, respectively. However, the highest necrosis was achieved with CUR (58.8%) and PTX (39.6%) as compared to the control (2.99%). This may be because of the development of apoptotic cells into secondary necrotic cells after a time. Secondary necrosis refers to late-stage apoptotic cells that phagocytes fail to engulf. Thus, secondary necrosis is a post-apoptotic event that eventually ceases to be metabolically active, loses membrane integrity, and releases cytoplasmic contents into the culture medium [35]. For normal HGF cells (Fig. 3B), the control cells showed higher viable cells (88.6%), followed by CUR (86.4%) and PTX (81%). The highest early and late apoptosis was achieved with PTX, while the highest necrosis was achieved with CUR (9.68%) compared to PTX (6.94%) and the control (2.69%).

In conclusion, when used at their IC_{50} values, CUR presents a significant amount of apoptosis and necrosis in oral cell lines, resulting in DNA damage and preventing cancer development. Some data have reported that CUR has a biphasic action (acts on the proteasome), with activation at lower doses and suppression at higher doses. Since suppression

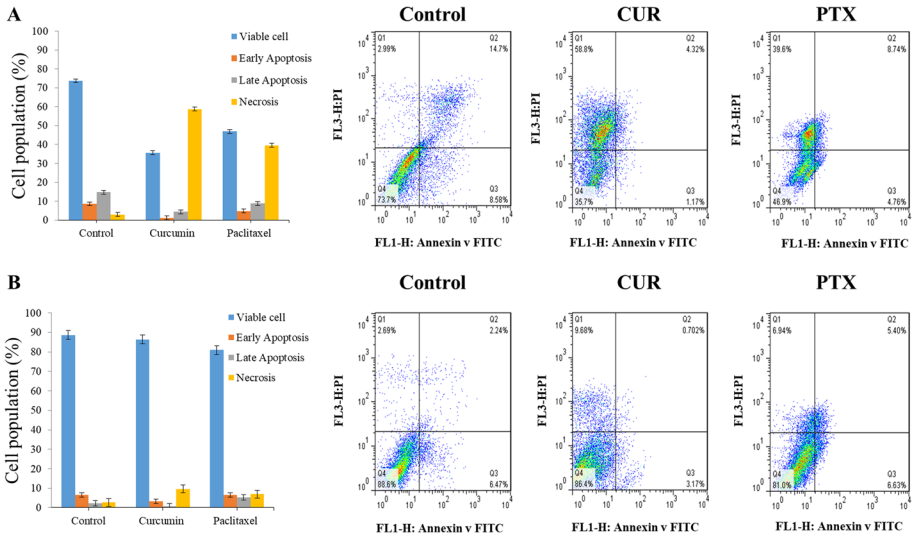

Fig. 3 Flow cytometry of TSCCF cancer cells (**A**) and HGF normal cells (**B**) after 72 h treatment at the IC_{50} levels for control cells, cells treated with CUR, and cells treated with PTX, respectively

of the proteasome leads to apoptosis, and its activation leads to cell survival, maybe CUR can lead to apoptosis or survival based on the dose used. Further, CUR at various doses also may impact the cell death type: low doses lead to apoptosis and oxidative stress, while high doses lead to necrotic cell death and reduced production of ROS and ATP [36]. This result is similar to that of the MCF-10F cell line, in which PTX alone induced only 7.3% necrosis with no signs of necrosis events for the MDA-MB-231 cells. On the other hand, a combination of CUR and PTX induced 35.3% and 19.4% necrosis in the MCF-10F and MDA-MB-231 cell lines, respectively [17].

The cells treated with CUR and PTX were stained with V/PI and then analyzed by flow cytometry (Fig. 3). Apoptotic treatment caused several morphological changes at variable time intervals. Therefore, determining the time interval that defines the type of cell death because the cells do not simultaneously respond to apoptotic conditions is essential [33]. Several studies have shown that cell death patterns shift from apoptosis to necrosis depending on various factors, including the nature of drugs, cell genotype, drug concentration, light fluorescence, or both. However, apoptosis and necrosis cell death depends on intracellular energy levels and is characterized by a combination of biochemical and morphological features resulting in identifying the death mechanism type involved [33, 37].

ROS Analyses

ROS plays an essential role in maintaining redox balance [38]. Cancer cells maintain their production of ROS by suppressing levels of antioxidants. Cancer cells have higher levels of ROS than normal cells because of their higher metabolic rate. Thus, the production of ROS induced by anti-cancer agents is known to be correlated with apoptosis [39]. DCFH-DA is a fluorescent probe used to determine the ROS generation quantity to explore ROS' role in stimulating apoptosis using the IC_{50} concentration of CUR and PTX. Figures 4 and 5 show no significant ROS stimulation in TSCCF and HGF cell lines treated with CUR and PTX (DCFH, 45.9, 63.9%, and 5.79, 32.2%, respectively) as compared to the control (DCFH, 79.5%, and 88.9%, respectively). A previous study has shown that CUR, either alone or in co-application with anti-cancer drugs, can efficiently induce apoptosis. Nonetheless, the main mechanisms of CUR-induced cytotoxicity remain controversial because of changeable anti- and pro-apoptotic signaling pathways in various cell types [13]. However, the exact mechanism of CUR needs further analysis. It has been reported that *Caesalpinia sappan* L. heartwood ethanolic extract-treated 4T1 breast cancer cells at different concentrations (12.5, 25, and 37.5 μg mL^{-1}) can significantly reduce intracellular ROS levels ($*p < 0.05$) [40].

Wide-ranging research over many years has tried to determine the molecular mechanisms of CUR. However, several experimental pieces of evidence show that CUR is a main constituent in preventing cancer progression by regulating gene expression or activating signaling pathways. CUR regulates the expression of proteins related to apoptosis (Bcl-2, Caspases, and Fas), cell cycle proteins (cyclin D1), and transcription factors (NF-κB, AP-1, and STAT) and their signaling pathways. Based on these facts, CUR can potentially prevent and treat various diseases, including cancer [41, 42]. For example, CUR could induce cell apoptosis by reducing Bcl-2 expression and activation of caspase-3 [43]. The results from Banerjee et al. [44] suggest that CUR could decrease the expression of anti-apoptotic proteins such as BCL-XL, MCL-1, and BCL-2 and increase the expression of pro-apoptotic proteins such as BAK and BID in treated cancer cells. This finding has been confirmed by Li et al. [45], Scott and Loo [46], and Narayan

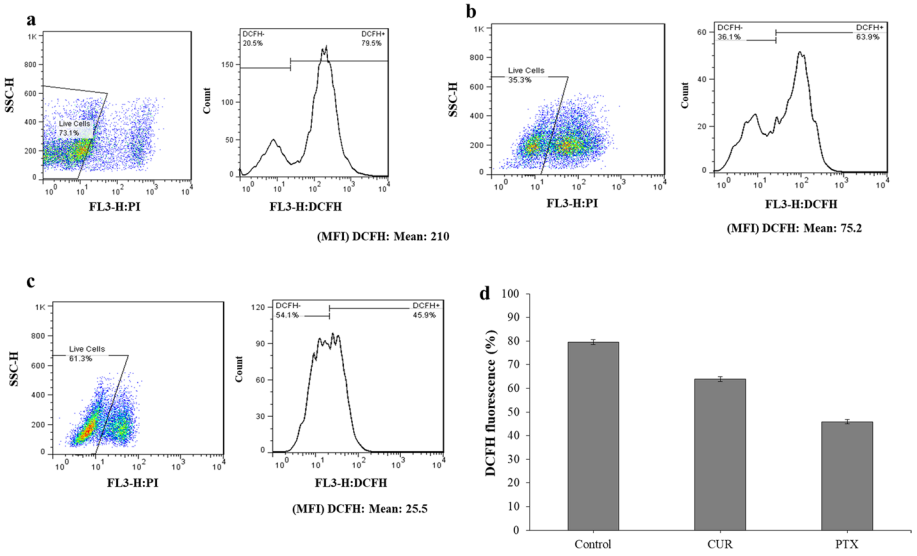

Fig. 4 Flow cytometry analysis of TSCCF cancer cells for the detection of ROS in different treatments **a** control; **b** CUR; **c** PTX; **d** DCFH fluorescent (%). DCFDA-: negative cells, DCFDA +: positive cells. Note the significantly lower production of ROS after TSCCF with CUR and PTX compared to the control group. Data are expressed as means ± SD. ***$P \leq 0.0001$

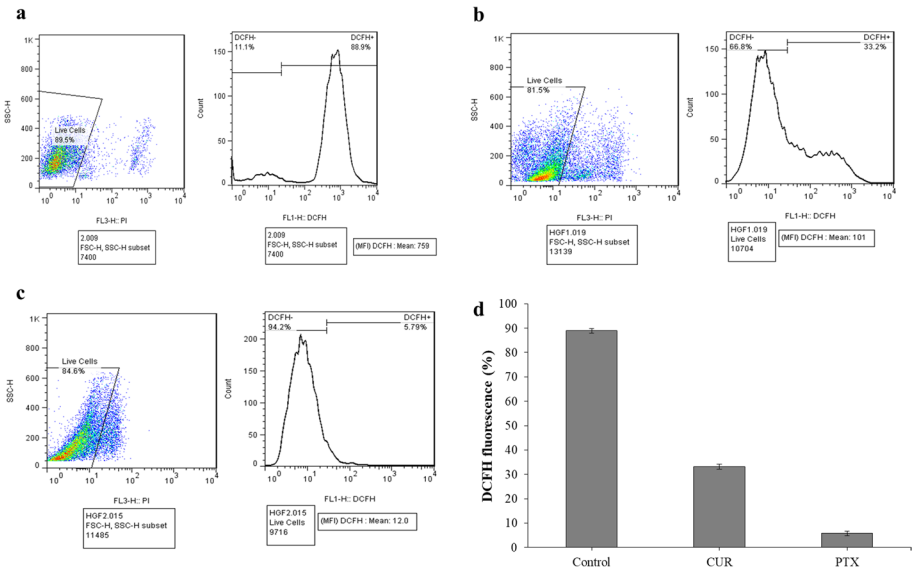

Fig. 5 Flow cytometry analysis of HGF normal cells for the detection of ROS in different treatments **a** control; **b** CUR; **c** PTX; **d** DCFH fluorescent (%). DCFDA-: negative cells, DCFDA +: positive cells. Note the significantly lower production of ROS after TSCCF with CUR and PTX compared to the control group. Data are expressed as means ± SD. ***$P \leq 0.0001$

[47] that reported CUR promotes apoptosis of leukemia cancer through a boost in caspase-3 and Bax expression and a decrease in Bcl-2 expression. Further, Fetoni et al. [48] reported that CUR in a preclinical study increased the heme oxygenase-1 (modulator of cytoprotection) expression.

In conclusion, the cytotoxicity of CUR and PTX is not through the ROS or senescence pathway. However, CUR and PTX may be induced apoptosis in TSCCF and HGF cell lines via the mitochondria-related apoptotic pathway. Therefore, additional in vitro and in vivo studies are needed to determine the CUR anti-cancer mechanism (angiogenesis pathway, transduction pathway, and apoptosis pathway) and its side effects to evaluate its therapeutic capacity in future oral cancer treatment.

Curcumin Characterization

NMR Results

The residual solvent signals for DMSO (2.5 ppm) and water (3.38 ppm) were excluded. As observed in Fig. 6, the signal for 6H has shifted to δ 3.84 ppm as a singlet corresponding to two methoxy groups, the 1H and H-1 signal is observed in δ 6.06 ppm as a singlet, and the H-3 signal is observed in δ 6.74 ppm as a doublet ($J = 18$ Hz), the 9H and H-9 signal is observed in δ 6.82 as a doublet ($J = 12$ Hz). The signals for the aromatic hydrogens are shifted from δ 6.67 to 7.15 ppm [49, 50]. The 10 H and H-10 signal is observed in δ 7.14 as a doublet ($J = 12$ Hz), and the 2H signal is observed in δ 7.32 as a single; a doublet at δ 7.50–7.55 ppm also appeared for hydrogen atom adjacent to the benzene ring in CUR [51]. The doublet signal for H4 and H-4 has shifted to δ 7.54 ppm (2H, $J = 18$ Hz). A single sharp peak at 9.65 ppm indicated the hydroxyl groups of CUR. Several studies have reported all peaks detected in CUR using 1H NMR, confirming the CUR purity [50, 52, 53].

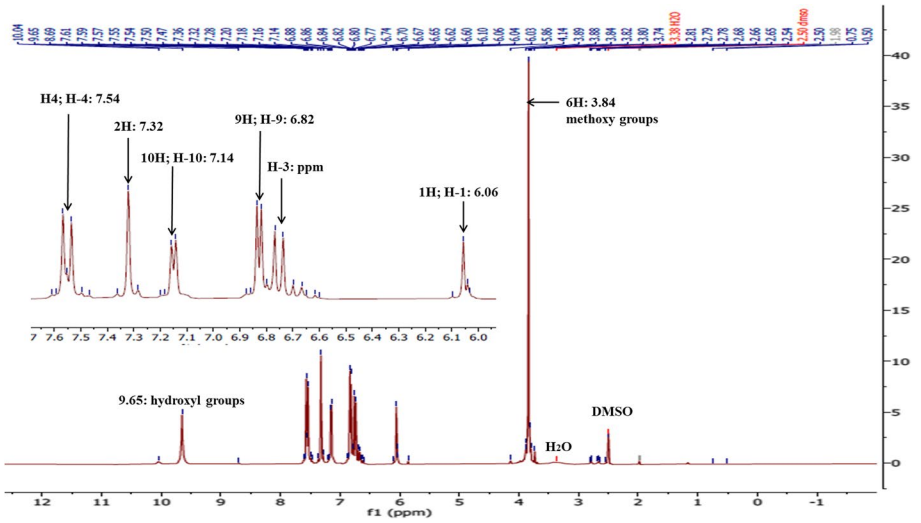

Fig. 6 Nuclear magnetic resonance (NMR) spectra of curcumin (.[1]H NMR (500 MHz, DMSO-d6)

Conclusion

This study's findings point to the remarkable fact that these compounds are selective toward cancer cells and less effective on normal cells, thus reducing systemic toxicity. However, these treatments did not induce any apoptosis characteristic of the normal cell line, indicating a protective effect and specifically targeting cancer cells. From the preliminary report, the conclusion can be reached that CUR shows good anti-cancer effects against oral cancer. Nonetheless, additional research is required to clarify the precise molecular mechanism involved in inhibiting cell viability, examine the activity of CUR using different cell lines, and apply CUR to animal models to determine whether CUR has potential therapeutic effects on oral cancer.

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Author Contribution Hanaa Ali Hussein: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper.

Fatin L. Khaphi: conceived and designed the experiments; contributed reagents, materials, analysis tools, or data; analyzed and interpreted the data; wrote the paper.

Data Availability The published article includes all data generated or analyzed during this study. The study's raw data are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval and Consent to Participate Inapplicable.

Patient Consent for Publication Inapplicable.

Competing Interests The authors declare no competing interests.

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