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Caffeate derivatives induce apoptosis in COLO 205 human colorectal carcinoma cells through Fas- and mitochondria-mediated pathways

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ABSTRACT

The objective of this study was to investigate the anticancer activity of caffeate derivatives in human cancer cells. Our results demonstrate that caffeate derivatives decreased the population growth of COLO 205, assessed using the MTT assay. However, caffeate derivatives, at the concentrations used in this study (0–250 μM) did not affect the viability of HepG2, Huh7, PLC5, and SK-Hep-1 cells. Flow cytometric analysis of COLO 205 cells exposed to decyl caffeate showed that the number of apoptotic cells increased in a time- and dose-dependent manner. Western blot analysis revealed that decyl caffeate stimulated an increase in protein expression levels of p53, Fas, FasL, AIF, and Apaf-1. Additionally, treatment with decyl caffeate changed the expression levels of Bcl-2 family members and subsequently induced the activation of caspase-12, caspase-9, and caspase-3, which was followed by cleavage of PARP. Our findings highlight the chemopreventive potential of decyl caffeate.

1. Introduction

Over the last two decades, many studies have focused on understanding the molecular mechanisms of apoptosis induced by naturally-occurring bioactive compounds in human cancer cells. This research has led to progress in novel strategies for cancer therapy (Araújo, Gonçalves, & Martel, 2011; Onori et al., 2009). Apoptosis, or programmed cell death, can be activated through two main pathways, including a mitochondrion-dependent pathway (the intrinsic pathway) and the death receptor-dependent pathway (the extrinsic pathway) (Thornberry et al., 1997). Moreover, the endoplasmic reticulum stress pathway has been shown to play an important role in cell apoptosis (Reuter, Eifes, Dicato, Aggarwal, & Diederich, 2008). In the mitochondria, apoptotic signals are regulated by Bcl-2 family members, such as the anti-apoptotic members Bcl-2 and Bcl-xL, and the pro-apoptotic members Bax, Bad, and Bak (Reuter et al., 2008; Yanez et al., 2004). Many models of apoptosis have demonstrated that mitochondria undergo a permeability transition, which causes a loss of mitochondrial membrane potential and a loss of cytochrome *c* from the mitochondria into the cytosol. This process precedes caspase activation (Madesh, Antonsson, Srinivasula, Alnemri, & Hajnoczky, 2002; Zamzami, Metivier, & Kroemer, 2000). The extrinsic pathway involves the death-inducing signalling complex, which includes a key regulator of apoptosis, the Fas ligand, binding to the Fas receptor.

Caffeic acid esters are a component of propolis and are reported to have a broad spectrum of biological effects, such as anti-tumour, antioxidant, and anti-inflammatory activities (Burdock, 1998). Uwai et al. (2008) demonstrated that the alkyl side-chain of caffeate derivatives decreased lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in RAW264.7 macrophages. The results from an MTT assay showed that two novel cytotoxic benzofuran derivatives from Brazilian propolis decreased the population growth of murine colon 26-L5 carcinoma and human HT-1080

Abbreviations: AIF, anti-apoptosis-inducing factor; Apaf-1, anti-apoptotic protease activating factor-1; CAPE, caffeic acid phenylethyl ester; DMSO, dimethylsulphoxide; FLIP, FLICE-inhibitory protein; ΔΨm, mitochondrial membrane potential; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PARP, poly(ADP-ribose) polymerase; PBS, phosphate buffered saline; PI, propidium iodide; PVDF, polyvinyldifluoride; ROS, reactive oxygen species; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

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fibrosarcoma cells (Banskota, Tezuka, Midorikawa, Matsushige, & Kadota, 2000). Banskota et al. (2002) established that the IC_{50} (inhibits growth of 50%) values of benzyl and phenethyl caffeates on colon 26-L5 carcinoma cells were 0.288 and 1.76 μM, respectively. Kudugunti et al. (2010) showed that phenethyl caffeate, caffeic acid phenylethyl ester (CAPE), induces apoptosis in SK-MEL-28 human melanoma cells through quinone formation, reactive oxygen species (ROS) formation, intracellular GSH depletion, and induced mitochondrial toxicity. Zou et al. (2010) demonstrated that the caffeate derivative compound (*E*)-1-(4-(3,4-dichlorobenzyl)piperazin-1-yl)-3-(4-(4-ethoxybenzyloxy)-3,5-dimethoxyphenyl)prop-2-en-1-one had significant and selective cytotoxicity in KB, BEL7404, K562, and Eca109 human cancer cell lines. However, studies demonstrating the anticancer effects of caffeate derivatives, including octyl caffeate (**1**), phenylpropyl caffeate (**2**), and decyl caffeate (**3**), in human cancer cells remain inconclusive.

The objective of this study was to investigate the anticancer effects of caffeate derivatives in human cancer cells. In this study, various human cancer cells (including HepG2, Huh7, PLC5, SK-Hep-1, and COLO 205 cells) were used to investigate anticancer activity *in vitro*. Specifically, the anticancer effect of caffeate derivatives on apoptotic pathways in human cancer cells was investigated.

2. Materials and methods

2.1. Materials

Caffeic acid, MTT dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], propidium iodide (PI), and sodium bicarbonate were purchased from Sigma Chemical Co. (St. Louis, MO). Dimethylsulphoxide (DMSO) was purchased from the Merck Co. (Darmstadt, Germany). Dulbecco's modified Eagle's medium, foetal bovine serum, L-glutamine, non-essential amino acids, sodium pyruvate, and the antibiotic mixture (penicillin–streptomycin) were purchased from Invitrogen (Carlsbad, CA). Anti-β-actin and anti-caspase-12 antibodies were purchased from Cell Signaling Technology (Beverly, MA). Anti-Bad, anti-p53, anti-Fas, and anti-caspase-3 antibodies were purchased from BD Biosciences (San Jose, CA). Anti-FasL antibody was purchased from BioVision (Mountain View, CA). Anti-Bax, anti-Bcl-2, anti-apoptosis-inducing factor (AIF), anti-apoptotic protease activating factor-1 (Apaf-1), anti-FLICE-inhibitory protein (FLIP), anti-caspase-9, and anti-poly (ADP-ribose) polymerase (PARP) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-rabbit or anti-mouse secondary horseradish peroxidase antibodies were purchased from Bethyl Laboratories (Montgomery, TX). Protein molecular mass markers were obtained from Pharmacia Biotech (Saclay, France). Polyvinyldifluoride (PVDF) membranes for Western blotting were obtained from Perkin Elmer Life Sciences (Boston, MA). All other chemicals were reagent grade.

2.2. Synthesis of caffeate derivatives

Compounds were obtained from the following method of ester binding coupling (Fig. 1). Caffeic acid (200 mg) and 4 mL $SOCl_2$ dissolved in dry CH_2Cl_2 (10 mL) were heated under reflux for 4 h. The reaction solvent and $SOCl_2$ was removed under vacuum, and then ROH (1.2 equiv) in triethylamine (0.08 mL) was added dropwise under dry conditions. The reaction mixture was stirred for 24 h at ambient temperature, and then was evaporated under vacuum. The residue was partitioned between ethyl acetate (AcOEt) and H_2O, successively; the AcOEt layer was washed with 3 N aqueous HCl and 10% $NaHCO_3$ (aq.), dried over $MgSO_4$ and concentrated under vacuum. The product was further purified by column chromatography on silica gel. The final products (60–65% yield) were recrystallised from acetone to obtain pure crystals. ^1H NMR spectra were recorded on a Bruker Avance 500 spectrometer. Electron impact mass spectra (EIMS) were determined on a Finnigan TSQ-46C mass spectrometer. IR spectra were recorded on a Nicolet Magna-IR 550 spectrophotometer.

Octyl caffeate (**1**): White solid, mp 98–100 °C, IR ν_{max} (cm^{-1}) 3488, 3340, 1675, 1630, 1274, 1181, 972, 812. ^1H NMR (CD_3COCD_3) δ 0.85 (3H, t, J = 6.7 Hz), 1.26 (10H, m), 1.67 (2H, quin J = 6.7 Hz), 4.16 (2H, t, J = 6.7 Hz), 6.23, 7.54 (each 1H, d, J = 15.9 Hz), 6.84 (1H, d, J = 8.2 Hz), 6.96 (1H, dd, J = 8.2, 2.0 Hz), 7.06 (1H, d, J = 2.0 Hz), 8.26 (2H, br s, –OH). EI-MS *m/z* (%): 292 (M^+, 27), 180 (100), 163 (47), 145 (8), 136 (18), 134 (12), 89 (13).

Phenylpropyl caffeate (**2**): White solid, mp 102–103 °C, IR ν_{max} (cm^{-1}) 3482, 3327, 1671, 1629, 1597, 1179, 973, 809, 696. ^1H NMR ($CDCl_3$) δ 2.01 (2H, quin J = 6.8 Hz), 2.72, 4.20 (each 2H, t, J = 6.8 Hz), 6.25, 7.55 (each 1H, d, J = 15.9 Hz), 6.85 (1H, d, J = 8.2 hz), 6.98 (1H, dd, J = 8.2, 1.8 Hz), 7.08 (1H, d, J = 1.8 Hz), 7.10–7.30 (5H, m). EI-MS *m/z* (%): 298 (M^+, 18), 180 (100), 163 (19), 135 (8), 118 (30), 117 (30), 91 (24).

Decyl caffeate (**3**): White solid, mp 108–109 °C, IR ν_{max} (cm^{-1}) 3484, 3326, 1678, 1629, 1597, 1527, 1275, 1181, 972, 859, 812. ^1H NMR ($CDCl_3$) δ 0.85 (3H, t, J = 6.7 Hz), 1.24 (14H, m), 1.66 (2H, quin J = 6.7 Hz), 4.16 (2H, t, J = 6.7 Hz), 5.89, 6.01 (each 1H, br s, –OH), 6.24, 7.55 (each 1H, d, J = 16.0 Hz), 6.85 (1H, d, J = 8.2 Hz), 6.98 (1H, dd, J = 8.2, 2.0 Hz), 7.03 (1H, d, J = 2.0 Hz). EI-MS *m/z* (%): 320 (M^+, 48), 180 (100), 163 (62), 145 (9), 136 (28), 134 (15), 89 (22).

2.3. Cell culture

Human hepatoblastoma cells (HepG2 cells) were obtained from the Bioresource Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsinchu, Taiwan). Human hepatocellular carcinoma cells (Huh7, PLC5, and SK-Hep-1 cells) and human colorectal carcinoma cells (COLO 205 cells) were obtained from the American Type Culture Collection (ATCC, Bethesda, MD). HepG2, Huh7, PLC5, and SK-Hep-1 cells were grown in 90% Dulbecco's modified Eagle's medium, supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 100 units/mL penicillin, and 100 μg/mL streptomycin. COLO 205 cells were grown in 90% RPMI 1640 medium supplemented with 10% foetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured at 37 °C in a humidified 5% CO_2 incubator.

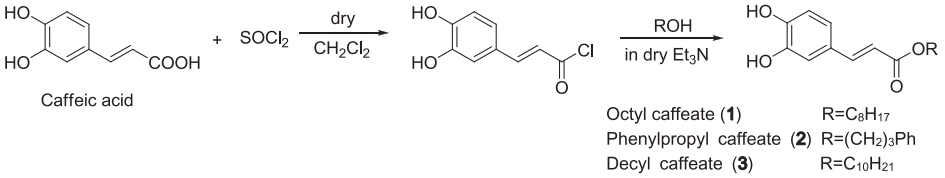

Fig. 1. The synthetic procedure and chemical structures of caffeate derivatives.

2.4. Cell viability by MTT assay

The MTT assay was performed according to the method of Mosmann (1983). Cancer cells were plated in 96-well microtitre plates at a density of 1×10^4 cells/well. After 24 h, the culture medium was replaced with 200 μL serial dilutions (0–250 μM) of caffeic acid derivatives, and the cells were incubated for 48 h. The final concentration of the solvent was less than 0.1% in cell culture medium. The culture medium was removed and replaced with 90 μL of fresh culture medium. Ten microlitres of sterile filtered MTT solution (5 mg/mL) in phosphate buffered saline (PBS, pH 7.4) were added to each well, reaching a final concentration of 0.5 mg MTT/mL. After 5 h, the unreacted dye was removed, the insoluble formazan crystals were dissolved in 200 μL/well of DMSO, and the plate was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 570 nm. The relative cell viability (%) related to control wells containing cell culture medium without samples was calculated as:

$$100 \times A_{570\ \mathrm{nm}}\ (\mathrm{sample}) / A_{570\ \mathrm{nm}}\ (\mathrm{control}).$$

2.5. Nuclear staining with PI

Apoptosis was evaluated by staining with propidium iodide (PI). Cells were stimulated with 0–100 μM of caffeic acid derivatives for 24 and 48 h. PI-stained cells were fixed with 80% ethanol for 30 min and incubated with 40 μg/mL PI solution for 30 min in the dark. The nuclear morphology of the cells was examined by fluorescence microscopy (Olympus, Tokyo, Japan). Typical apoptotic changes included chromatin condensation, chromatin compaction along the periphery of the nucleus, and segmentation of the nucleus.

2.6. Mitochondrial membrane potential ($\Delta\Psi m$) assay

Mitochondrial membrane potential assay was performed using the JC-1 mitochondrial membrane potential assay kit (Cayman Chemical Co., Ann Arbor, MI). Cells were seeded in 6-well plates. After 24 h, the cells were treated with 0–25 μM of caffeic acid derivatives for 6 and 12 h. The cells were labelled with JC-1 according to the manufacturer's instructions. The cells were resuspended in adequate amounts of the same solution and were analysed by FLUOstar galaxy fluorescence plate reader with an excitation wavelength of 560 nm and an emission wavelength of 595 nm for red fluorescence. Apoptotic cells will generate a lower reading of red fluorescence, and the changes in the mitochondrial membrane potential ($\Delta\Psi m$) can most accurately be assessed by comparing the red fluorescence of untreated cells and cells treated with caffeic acid derivatives. The morphology of the cells was examined by fluorescence microscopy (Olympus, Tokyo, Japan).

2.7. Western blot analysis

Cells (1×10^7 cells/10 cm dish) were incubated with 25 μM of caffeate derivatives for 0, 1, 3, 6, 9, 12, and 24 h. Cells were collected and lysed in ice-cold lysis buffer [20 mM tris–HCl (pH 7.4), 2 mM EDTA, 500 μM sodium orthovanadate, 1% Triton X-100, 0.1% SDS, 10 mM NaF, 10 μg/mL leupeptin, and 1 mM PMSF]. The p53, Fas, FasL, AIF, Apaf-1, FLIP, Bcl-2, Bax, Bad, caspase-12, caspase-9, caspase-3, PARP, and β-actin proteins were assessed in COLO 205 cells. The protein concentration of the extracts was estimated with the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as the standard. Total proteins (50–60 μg) were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) using a 12% polyacrylamide gel. The proteins in the gel were transferred to a PVDF membrane. The membrane was blocked with 5% skim milk in PBST (0.05% v/v Tween-20 in PBS, pH 7.2) for 1 h. Membranes were incubated with primary antibody at 4 °C overnight and then with secondary antibody for 1 h. Membranes were washed three times in PBST for 10 min between each step. The signal was detected using enhanced chemiluminescence (ECL; Perkin Elmer Life Sciences).

2.8. Statistical analysis

Statistical analysis was performed using the SAS software. Analysis of variance was performed. Significant differences ($p < 0.05$) between the means were determined by Duncan's multiple range tests. Each treatment was performed in triplicate.

3. Results and discussion

3.1. Effect of caffeate derivatives on cell population growth in COLO 205 cells

The chemical structures of the caffeate derivatives [including octyl caffeate (**1**), phenylpropyl caffeate (**2**), and decyl caffeate (**3**)] tested in the present study are shown in Fig. 1. In the present study, various human cancer cells (including HepG2, Huh7, PLC5, SK-Hep-1, and COLO 205 cells) were used for *in vitro* evaluation of anticancer activity. The effect of octyl caffeate (**1**), phenylpropyl caffeate (**2**), and decyl caffeate (**3**) on cell population growth is shown in Fig. 2. The results show that addition of octyl caffeate, phenylpropyl caffeate, and decyl caffeate to the growth medium decreased the population growth of human colorectal carcinoma cells, COLO 205. When the cells were treated with 250 μM of caffeic acid derivatives for 48 h, the cell viability of octyl caffeate, phenylpropyl caffeate, and decyl caffeate on COLO 205 cells was 70.9 ± 7.4%, 70.4 ± 8.2%, and 48.8 ± 8.7%, respectively. However, decyl caffeate had the strongest growth inhibition of COLO 205 cells. Our results also indicate that the cell viability (%) of caffeic acid (250 μM, 48 h) on COLO 205 cells was 78.8 ± 2.7% (data is not shown in figure/table). An examination of cell viability in the presence of caffeate derivatives in HepG2, Huh7, PLC5, and SK-Hep-1 cells indicates that the concentrations (0–250 μM) of the compounds used in this study did not affect the viability of the HepG2, Huh7, PLC5, and SK-Hep-1 cells (data not shown). Banskota et al. (2002) demonstrated that some caffeate derivatives (benzyl caffeate, phenethyl caffeate, and cinnamyl caffeate) have a strong inhibitory effect on the population growth of cancer cell lines (including human HT-1080, human A-549, murine colon 26-L5, and murine B16-BL6 cells). Omene, Wu, and Frenkel (2011) showed that CAPE decreased the population growth of breast cancer stem cells. Serafim et al. (2011) indicated that lipophilic caffeic and ferulic acid derivatives inhibited cell proliferation and induced cell apoptosis in MCF-7 human breast cancer cells.

3.2. Effect of decyl caffeate on cell apoptosis in COLO 205 cells

In the present study, COLO 205 cells were selected for studying the induction of decyl caffeate on cell apoptosis. The results are shown in Fig. 3. Addition of decyl caffeate to COLO 205 cells resulted in a marked increase in the level of accumulation of the sub-G1 phase (apoptotic cells) in a time- and dose-dependent manner. The effect of decyl caffeate on cell morphology in COLO 205 cells is shown in Fig. 4. Classical apoptotic cells were identified after decyl caffeate treatment by identification of cell shrinkage, membrane blebbing, and apoptotic body formation (Fig. 4A). The nuclear morphology of untreated and treated cells stained with PI is shown in Fig. 4B. PI staining showed apoptotic bodies when cells were treated with 100 μM of decyl caffeate for 48 h. Serafim

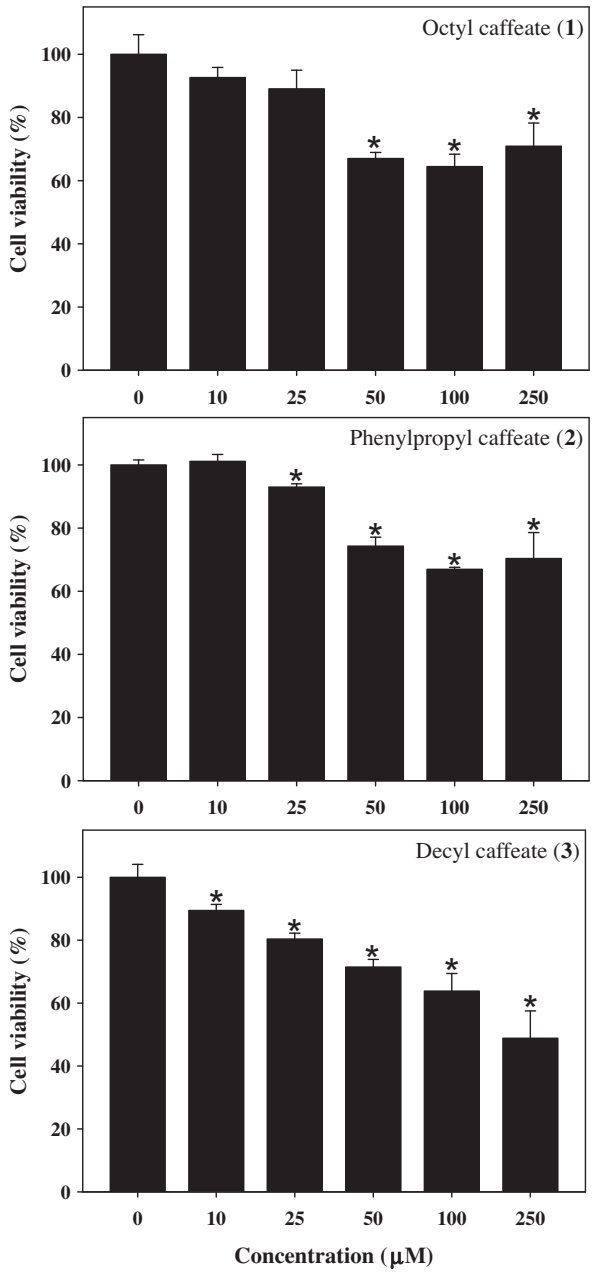

Fig. 2. Effect of caffeate derivatives on cell viability in COLO 205 cells. Cells were treated with 0–250 μM of caffeic acid derivatives for 48 h. The reported values are the means ± SD ($n = 3$). $^{*}p < 0.05$ shows statistical significance when compared with the control.

et al. (2011) indicated that the addition of caffeic acid derivatives-caffeoylhexylamide to MDA-MB-231 and HS578T human breast cancer cells resulted in a marked increase in the level of accumulation of the sub-G1 phase (apoptotic cells) in a time-dependent manner. Nagaoka et al. (2003) indicated that caffeate derivatives (4-phenylbutyl caffeate, 8-phenyl-7-octenyl caffeate, 2-cyclohexylethyl caffeate, and *n*-octyl caffeate) significantly decreased the number of tumour nodules in their lung metastasis formation.

3.3. Effect of decyl caffeate on mitochondrial membrane potential ($\Delta\Psi m$) in COLO 205 cells

Functional alterations of mitochondria have been shown to play an important role in cell apoptosis. The effect of decyl caffeate on

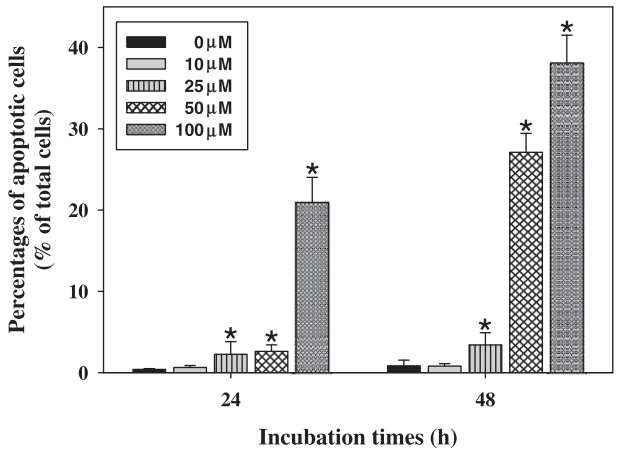

Fig. 3. Flow cytometric analysis of decyl caffeate-mediated cell apoptosis in COLO 205 cells. Percentages of apoptotic cells were calculated by WinMDI 2.9 software. The reported values are the means ± SD ($n = 3$). $^{*}p < 0.05$ is significantly different compared with the control.

mitochondrial membrane potential ($\Delta\Psi m$) in COLO 205 cells is shown in Fig. 5. Cell morphology indicated that non-apoptotic cells with healthy mitochondria appear as red fluorescent cells, and apoptotic cells appear as green fluorescent cells. COLO 205 cells showed a significant ($p < 0.05$) decrease in red fluorescence intensity when treated with 0–25 μM of decyl caffeate for 6 and 12 h. Chen et al. (2008) demonstrated that treatment of BxPC-3 human pancreatic cancer cells with CAPE causes the loss of mitochondria membrane potential.

3.4. Decyl caffeate induces apoptosis via a Fas- and mitochondrial-mediated pathway

Apoptosis may be initiated through the regulation of death receptors located on the cell surface or through an intrinsic pathway, which includes the release of apoptotic signals from the mitochondria (Vermeulen, Van Bockstaele, & Berneman, 2005). The effect of decyl caffeate on the expression of p53, Fas, FasL, AIF, Apaf-1, FLIP, Bcl-2, Bax, Bad, caspase-12, caspase-9, caspase-3, and PARP in COLO 205 cells was measured by Western blot analysis (Fig. 6). COLO 205 cells were treated with 25 μM of decyl caffeate for 0, 1, 3, 6, 9, 12, and 24 h. p53 (also known as protein 53 or tumour protein 53) is a tumour suppressor gene that helps regulate cell cycle and cell apoptosis (Shen & White, 2001). Fas and its receptor Fas ligand (FasL) play an important role in regulating the induction of apoptosis in diverse cell types and tissues (Nagata & Golstein, 1995). After being treated with decyl caffeate, the maximal level of p53 protein expression is at 3 h. Decyl caffeate (25 μM, 0–24 h) resulted in a significant increase in Fas and FasL expression. Lorenzo and Susin (2007) showed that anticancer drugs induce apoptosis *via* an AIF-mediated caspase-independent intrinsic pathway. Our results indicate that the protein expression levels of AIF increased after treatment with 25 μM of decyl caffeate for 0–24 h. The protein expression of Apaf-1 increased after treatment with 25 μM of decyl caffeate for 0–9 h. Apaf-1 has a central role in mitochondrial control of apoptosis and is an essential component of p53-regulated apoptosis (Zlobec, Vuong, & Compton, 2006). Li et al. (1997) showed that the upregulation of Apaf-1 leads to activation of caspase-9 and ultimately to apoptosis. Over the past years, many studies demonstrated a role for FLIP in death receptor-mediated signalling pathways (Thome & Tschopp, 2001; Yu & Shi, 2008). In the present study, the protein expression level of FLIP was decreased after treatment with 25 μM of decyl caffeate for 0–9 h.

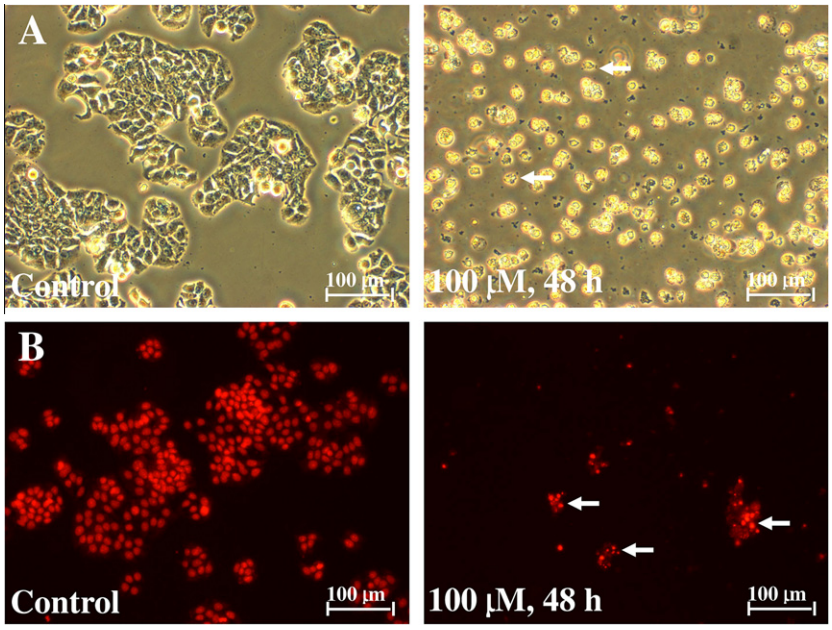

Fig. 4. Effect of decyl caffeate on cell morphology in COLO 205 cells. (A) Unstained and (B) stained with PI. Cells were treated with 100 μM of decyl caffeate for 48 h.

The Bcl-2 family plays a crucial role in apoptosis because it includes both anti-apoptotic members such as Bcl-2 and pro-apoptotic members such as Bax and Bad (Hunt & Evan, 2001). Expression of the anti-apoptotic protein Bcl-2 decreased after treatment with 25 μM of decyl caffeate for 0–9 h. Expression of the pro-apoptotic protein Bax, increased after treatment with 25 μM of decyl caffeate for 0–9 h but showed apparent reductions at 12 and 24 h after treatment. Moreover, the pro-apoptotic protein expression of Bad increased after treatment with decyl caffeate for 0–6 h. Activation of caspase-12 has been reported to play a key role in ER stress-mediated apoptosis (Szegezdi, Logue, Gorman, & Samali, 2006). Many reports have indicated that ER stress-induced apoptosis is mediated through mitochondria (Boya, Cohen, Zamzami, Vieira, & Kroemer, 2002). Treatment with decyl caffeate causes the activation of caspase-12, caspase-9, and caspase-3, which are associated with the degradation of PARP. These precede the onset of cell apoptosis. PARP is cleaved by caspase-3, which leads to DNA fragmentation and ultimately to apoptosis.

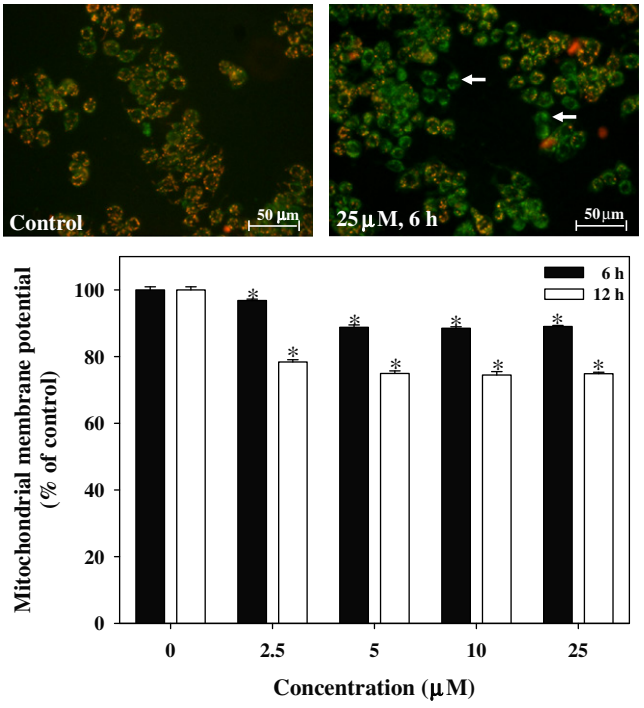

Fig. 5. Effect of decyl caffeate on mitochondrial membrane potential (ΔΨm) in COLO 205 cells. Cells were treated with 0–25 μM of decyl caffeate for 6 and 12 h. Reported values are the mean ± SD ($n = 3$). *Shows statistical significance when compared with the cells without treatment ($p < 0.05$).

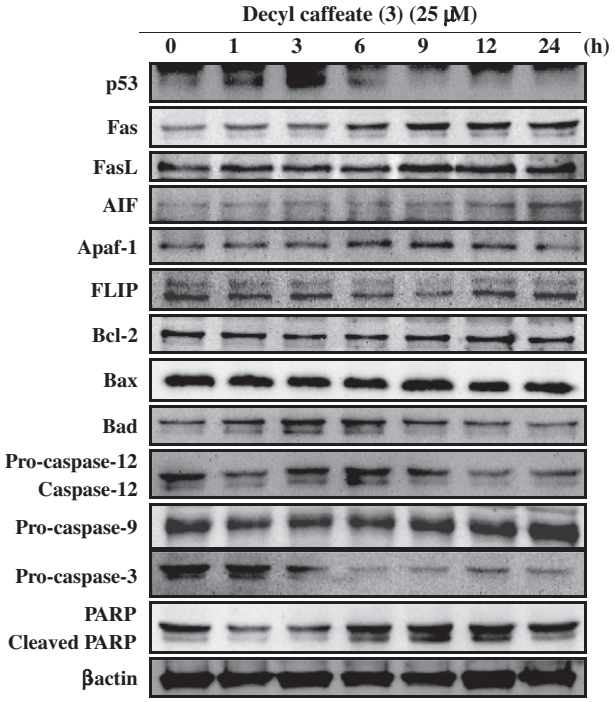

Fig. 6. Effect of decyl caffeate on protein expressions of p53, Fas, FasL, AIF, Apaf-1, FLIP, Bcl-2, Bax, Bad, caspase-12, caspase-9, caspase-3, and PARP in COLO 205 cells. Cells were treated with 25 μM of decyl caffeate for 0–24 h.

Treatment of cells with 25 μM of decyl caffeate induced PARP cleavage, which occurred 6 or 12 h after treatment. These results were further confirmed upon monitoring the cleavage of PARP, which is targeted by active caspase-3 (Debatin & Krammer, 2004). Many studies indicate that an increase in the ratio of Bax/Bcl-2 stimulates the release of cytochrome *c* from the mitochondria into the cytosol, upregulating caspase-9 expression, and binding to Apaf-1, which leads to the activation of caspase-3 and PARP cleavage (Bossy-Wetzel & Green, 1999; Pandey et al., 2000; Roy, Baliga, & Katiyar, 2005).

4. Conclusions

In conclusion, the present study showed that, of the caffeate derivatives prepared, decyl caffeate was the strongest growth inhibitor of COLO 205 cells. Treatment of COLO 205 cells with decyl caffeate caused loss of mitochondrial membrane potential. Western blot data revealed that decyl caffeate stimulates an increase in protein expressions of p53, Fas, FasL, AIF, and Apaf-1. Additionally, treatment with decyl caffeate changed the expression levels of pro- and anti-apoptotic Bcl-2 family members and subsequently induced the activation of caspase-12, caspase-9, and caspase-3, which was followed by cleavage of PARP. These results demonstrate that decyl caffeate induces apoptosis in COLO 205 cells through both Fas- and mitochondria-mediated pathways.

Acknowledgements

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