

RESEARCH ARTICLE

Cytotoxic Effects of Phytochemicals from *Caesalpinia mimosoides* Lamk on Cervical Carcinoma Cell Lines through an Apoptotic Pathway

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Abstract

Background: Extracts of *Caesalpinia mimosoides* Lamk has been reported to possess anticancer effects, but the active ingredients and the anti-cancer mechanisms are still unknown. **Materials and Methods:** The effects of a *C mimosoides* Lamk extract on cell proliferation and apoptosis induction in human cervical carcinoma cell lines, namely HeLa, SiHa, and C33A, as well as in normal Vero cells, were investigated. **Results:** Treatment with 5 active fractions (F17-F21) of *C mimosoides* Lamk methanol extracts inhibited cell viability in a dose- and time-dependent manner. Neutral red assays indicated that treatment with F21 significantly decreased the viability of all cervical cancer cell lines compared to F21-treated normal cells. In addition, HPLC analysis revealed that F21 contained multiple phenolic compounds, namely gallic acid, caffeine, vanillic acid, ferulic acid and resveratrol. F21 had the lowest IC₅₀ and, therefore, a much higher cytotoxicity than F20, F17, F19, and F18 by 20-, 25-, 46- and 47- fold, respectively. Analysis of activation of the apoptosis pathway using a caspase 3/7 activity assay revealed that F21 treatment resulted in a considerable increase in caspase activation in all cancer cell lines tested. At the same concentration of F21, HeLa cells had the highest caspase activity (6.5-fold) compared to the control. **Conclusion:** *C mimosoides* Lamk may be of value as an alternative therapeutic agent, especially in combination with other compounds offering possible of synergy of action. Moreover, HPV- and non-HPV-related cervical cancer cells may differ in their responses to treatment regimens.

Keywords: Human papillomavirus (HPV) - cervical carcinoma - *Caesalpinia mimosoides* Lamk - phenolics - apoptosis

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Introduction

As compared to other cancers, the incidence of cervical carcinoma is relatively high (Arbyn et al., 2011). Certain human papillomavirus (HPV) types are carcinogenic in humans, and more than 99% of cervical cancers are associated with HPV infection. Genital HPV types are divided into high-, medium-, and low-risk groups based on the frequency of association with malignant tumors (Monsonogo et al., 2004). Radiotherapy is the treatment of choice for patients with locally advanced cervical carcinoma resulting in a progression-free survival rate of 70% (Rose, 2002). To increase local control rates, chemotherapeutic agents are being incorporated into treatment protocols. However, the success of chemotherapy for cervical cancer patients is hampered by the problem of drug-resistance. Therefore, it is of critical importance that an effective treatment for cervical cancer be developed.

Plant phytochemicals have been reported to prevent a number of diseases, including cancer, cardiovascular disease, infection and inflammation. *In vitro* studies have revealed that some phytochemicals, such as gallic acid (Gali et al., 1991; Ohno et al., 2001; Kim 2007; You et al., 2010; Lu et al., 2010), caffeine (Kawano et al., 2012), vanillic acid (Erdem et al., 2012), and ferulic acid (Karthikeyan et al., 2011; Serafim et al., 2011), are cytotoxic to cell lines. An *in vivo* model has shown that resveratrol not only has anti-inflammatory, anti-oxidative, anti-mutation, anti-proliferative, and anti-cell cycle properties but also induces apoptosis and has synergistic effects with chemotherapeutic drugs (Frampton et al., 2010; Hahnvajanawong et al., 2011). To date, many cytotoxic agents from natural products have been investigated for the discovery of novel anti-cancer drugs. *Caesalpinia mimosoides* Lamk is used as a fresh dietary vegetable indicating the possible safety of its future utilization. *C mimosoides* Lamk has been

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reported to exhibit anti-microbial (Chanwitheesuk et al., 2007) and anti-oxidant activities (Chanwitheesuk et al., 2005). Potential anti-inflammatory diterpenoids have been isolated from the roots of *C mimosoides Lamk* (Yodsaoue et al., 2010). Moreover, the cytotoxic effect of the methanolic crude extract from *C mimosoides Lamk* on oral cavity cell lines but not normal cells has been demonstrated, thereby suggesting a possible cancer-preventive role of *C mimosoides Lamk* (Daduang et al., 2011). There are few papers about the effect of bioactive compounds on cervical carcinoma cells. Therefore, the aim of this study was to examine if the active fractions from *C mimosoides Lamk* enhanced killing of both HPV-positive and HPV-negative cervical carcinoma cells. The effect of multiple and single phytochemical doses against these cells was also determined. A putative mechanism of the action of active fractions and induction of apoptosis by caspase 3/7 was studied in these cell lines.

Materials and Methods

Chemicals and reagents

Hexane, ethyl acetate, and methanol were purchased from S.C. Science Co., Ltd. (Thailand). Silica gel 60 and thin layer silica gel 60 F254 were purchased from Merck (Germany). Dulbecco's Modified Eagle's Medium-High Glucose (DMEM-HG), fetal bovine serum, penicillin-streptomycin, and trypsin-EDTA were obtained from Gibco BRL (Grand Island, NY, USA). Catechin, quercetin, ferulic acid, rutin, caffeine, gallic acid and neutral red were obtained from Sigma-Aldrich Co. LLC (USA). The Caspase-Glo 3,7 assay was obtained from Promega (Madison, USA).

Plant materials and extraction

Shoots and leaves of *C mimosoides Lamk* were harvested from Kamphangphet Province, Thailand in April 2011. The plants were identified as *C mimosoides Lamk* by Prof. Arunrat Chaveerach, Department of Biology, Faculty of Science, Khon Kaen University. Three kilograms of shoot and leaves were dried in a hot-air oven at 50°C and then ground to a fine powder. Five hundred grams of the obtained powder was extracted with distilled hexane at a ratio of 50 g powder per 200 ml of hexane and stirred overnight. The solution was filtered through Whatman no. 4 paper and evaporated using a rotary evaporator. The residue was re-extracted twice, and the supernatants were pooled and then evaporated by rotary evaporation at 40°C (Buchi R-240, Japan) under reduced pressure. This crude hexane extract (CHE) was kept for further analysis. The remaining residue was extracted with ethyl acetate (EtOAc) and methanol (MeOH) by the same procedure. Crude ethyl acetate extract (CEE) and crude methanol extract (CME) were also used for cell viability determination and cell proliferation assays.

Fractionation of crude extracts

Only CME inhibited the growth of cancer cell lines. Therefore, CME was applied to a silica gel (silica gel 60; 0.063-0.200 mm) column (diameter of 8 cm), and stepwise elution was performed by increasing polarity

of eluting solvent as follows: hexane, hexane:EtOAc, EtOAc, EtOAc:MeOH and MeOH. The percentage of solvents used is shown in Table 1. Each 75 ml fraction was collected and evaporated by rotary evaporation at 40°C, and the residues were used for bioassays.

Identification of compounds by high-performance liquid chromatography (HPLC)

One milligram of CME was re-dissolved in 1 ml of MeOH and filtered through polyvinylidene difluoride (Agela Technologies, USA). Each 20 µl fraction was injected into a HPLC column (Luna C18; 15 cm x3.0 mm; Phenomenex, USA) by using a modified method of Uckoo et al., 2012 (Uckoo et al., 2012). Gradient elution was conducted at a flow rate of 1.5 ml/min using methanol and 0.5% phosphoric acid at ratios of 5:95, 70:30, 90:10, and 5:95 at 0-17, 17-18, 18-20.5 and 20.5-25 min, respectively. The absorbance at 270 nm (Waters, UV 2479) was detected, and the peaks were analyzed by the Clarity program (Waters). Catechin, quercetin, ferulic acid, rutin, caffeine and gallic acid were used as standards.

Cell culture

HeLa (HPV-18-positive), SiHa (HPV-16-positive), and C33A (HPV-negative) cervical cancer cells as well as Vero (normal) kidney cells were placed in 25 cm² tissue culture flasks at 37°C with 5% CO₂ in DMEM-HG media supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin). Once the cells were approximately 70% confluent, they were trypsinized with 1 ml of 1X trypsin-EDTA, incubated at 37°C for 5 min and centrifuged at 1,800 rpm for 2 min. The supernatant was then removed, and 200 µl of seeding cells were resuspended in 5 ml of DMEM-HG media, and these seeding cells were considered mycoplasma-free cell lines.

Cell viability and IC₅₀ determination

The neutral red (NR) assay was used for cell viability determination. Cervical cancer and Vero cells (4x10³) in 100 µl of DMEM-HG media were used for cell viability tests. Each cell line was seeded into different 96-well plates. Each active fraction was dissolved in 20% DMSO. The cell lines were incubated with various final concentrations (20 to 120 µg/ml) of the active fractions at 37°C for 24 hr. The cells were then washed carefully with 250 µl of pre-warmed phosphate buffer saline (PBS). After removing the rinsing solution, the cells were incubated with 200 µl of NR media (99 ml of DMEM-HG plus 1 ml of pre-filtered 3.3 mg/ml NR) for 3 hr. The incubated cells were washed with 1XPBS and then lysed with 100 µl of NR desorb solution (absolute ethanol : glacial acetic acid : distilled water at a ratio 50:1:49). The plates were shaken for 30 min, and absorbance was measured at 540 nm using a spectrophotometer (Rayto RT-2100C, Germany). Cisplatin (20 µg/ml) was used as a positive control, and untreated cells were used as a negative control. An inhibition concentration at 50% (IC₅₀) was determined by plotting the percentage of cell viability versus drug concentrations. To study the anti-proliferative effect of single and mixed phenolic compounds, all cell lines

were also treated with gallic acid at the concentrations of 0, 10, 20, 40, 60, 80, 100 and 120 µg/ml, and the NR assay was performed following the same procedure. Each concentration of drug treatment was repeated in six wells for three independent experiments.

Morphological changes

As F21, a fraction from silica gel column chromatography, showed the highest cytotoxicity to HeLa, SiHa and C33A cells, the morphological changes of HeLa, SiHa and C33A cells treated with 40 µg/ml F21 and gallic acid for 24 hr were observed by using an inverted optical microscope at a magnification of 100X.

Apoptosis pathway analysis using a caspase activity assay

The caspase activity assay was performed using a test kit (Promega, USA). Cells (4×10^3) in 100 µl of media were seeded into different black 96-well plates. Active fractions dissolved in 20% DMSO at a final concentration of 100 µg/ml were added to the cell lines and incubated at 37°C for 24 hr.

The cells were incubated until room temperature was achieved and were then mixed with 100 µl of Caspase-Glo 3/7 reagents per well of a black 96-well plate also containing a blank, negative control and treated cells in culture media. Cisplatin was used as a positive control. The contents of the wells were gently mixed with a plate shaker at 300-500 rpm for 30 sec and incubated at room temperature for 1 hr. The luminescent signal of each sample was read with a SpectraMax L Luminescence microplate reader (Devices LLC, California, USA). The data were analyzed using Soft Max® Pro software (Devices LLC, California, USA).

Statistical analysis

Data were presented as the mean and standard error. Statistical significance was analyzed by one-way ANOVA using the GraphPad Prism software (version 5.0). P-values less than 0.05 ($p < 0.05$) were considered significant.

Results

Growth inhibitory effects of phytochemical fractions of *C. mimosoides* Lamk on human cervical cancer cells

The *C. mimosoides* Lamk CHE, CEE and CME yields were 29.65, 25.62 and 44.90 g, respectively. The effect of crude extracts on the proliferation of the HPV18-positive HeLa human cervical carcinoma cell line was first examined. Cells were treated with 200 µg/ml of crude extracts for 24 hr, and cell survival was then assessed using the NR assay. The anti-cancer drug, cisplatin, was used as a positive control. Only CME had an inhibitory effect on cell survival (data not shown). Thus, CME was selected for further partial purification by silica gel column chromatography. From 27 fractions obtained, 5 fractions (F17-F21) exhibited inhibitory effects against HeLa cells (Table 1). The IC_{50} values of F17-F21 compared to the IC_{50} value of cisplatin are shown in Table 2. Treatment of all cells with F21 resulted in a marked suppression of cell proliferation in a dose-dependent manner. The IC_{50} of F21 when treating HeLa, SiHa, C33A and Vero cells was < 20 ,

Table 1. The Fractions of *C. mimosoides* Lamk Obtained from Silica Gel Column Chromatography with Stepwise Elution and their Cytotoxic Activity Against HeLa cell Lines.

Fraction	Elution solvent	Cytotoxic activity
1	100% H	Negative
2	100% H	Negative
3	10% E/H	Negative
4	10% E/H	Negative
5	20% E/H	Negative
6	20% E/H	Negative
7	30% E/H	Negative
8	30% E/H	Negative
9	40% E/H	Negative
10	40% E/H	Negative
11	50% E/H	Negative
12	50% E/H	Negative
13	60% E/H	Negative
14	60% E/H	Negative
15	70% E/H	Negative
16	70% E/H	Negative
17	80% E/H	Positive
18	80% E/H	Positive
19	90% E/H	Positive
20	90% E/H	Positive
21	100% E	Positive
22	100% E	Negative
23	10% M/E	Negative
24	10% M/E	Negative
25	50% M/E	Negative
26	50% M/E	Negative
27	100% M	Negative

*H=Hexane, E=Ethyl acetate and M=Methanol. The fractions that had $IC_{50} < 120$ microgram/ml were called "active fractions"

Table 2. The IC_{50} of Cisplatin, 5 Active Fractions of *C. Mimosoides* Lamk and Gallic Acid Treated on the Cervical Cell Lines (HeLa, SiHa, and C33A) and Vero Cells

Cell line	IC_{50} (µg/ml)						Gallic Acid
	Cisplatin	F17	F18	F19	F20	F21	
HeLa	1.43	55.34	61.8	119.86	35.3	10	10
SiHa	16.6	> 100	> 100	106.6	> 100	20	37
C33A	4.39	55.04	48.6	< 20	10.6	12.36	20
Vero	5.86	> 100	93.4	> 100	74.3	52.05	> 100

20, 12.36 and 52.05 µg/ml, respectively. In additional data analysis, the cancer cell viability percentages were individually compared to that of Vero cells (Figure 1). The results demonstrated that treatment of HeLa and SiHa cells with F21 significantly decreased the percent cell viability ($p < 0.05$). For C33A cells, the percent cell viability was significantly decreased by both F19 and F21 ($p < 0.05$). F21 was subjected for further mechanistic studies because it had strong inhibitory effects against certain cancer cells (HeLa, SiHa and C33A) with few effects on normal cells.

High-performance liquid chromatography analysis

The phytochemical compositions of F17-F21 were analyzed by HPLC. The retention times of gallic acid, catechin, caffeine, vanillic acid, ferulic acid, rutin, resveratrol and quercetin standard compounds were

7.8, 11.3, 12.3, 13.4, 15.6, 16.6, 17.0 and 19.7 min, respectively. The retention time of F17 matched the retention time of caffeine. F18 was composed of gallic acid, caffeine, ferulic acid and resveratrol. F19, F20 and F21 contained gallic acid, caffeine, vanillic acid, ferulic acid and resveratrol, and the percentage differences are shown in Table 3.

Comparison of the IC₅₀ of F17-F21 with gallic acid

The HPLC profiles indicated that F19, F20 and F21 contained the same 4 types of phytochemicals. The majority of F21, which had the greatest inhibitory effects against all cancer cells tested, was gallic acid (68.49%). In additional experiments, we evaluated the effects of F21 compared to commercial gallic acid on 4 cell lines. The results showed that the IC₅₀ value of F21 was equal or less

Table 3. Percentage of HPLC peak area of F17-F21 Active Fractions Compared to Phytochemical Standards

Standard	Fractions				
	F17	F18	F19	F20	F21
Gallic acid	-	0.31	5.53	27.05	68.49
Catechin	-	-	-	-	-
Caffeine	78.79	76.14	48.12	6.34	3.44
Vanillic acid	-	-	7.37	10.86	5.59
Ferulic acid	-	0.58	28.25	42.97	20.63
Rutin	-	-	-	-	-
Resveratrol	-	2.41	2.72	-	1.85
Quercetin	-	-	-	-	-

- = no peak detected

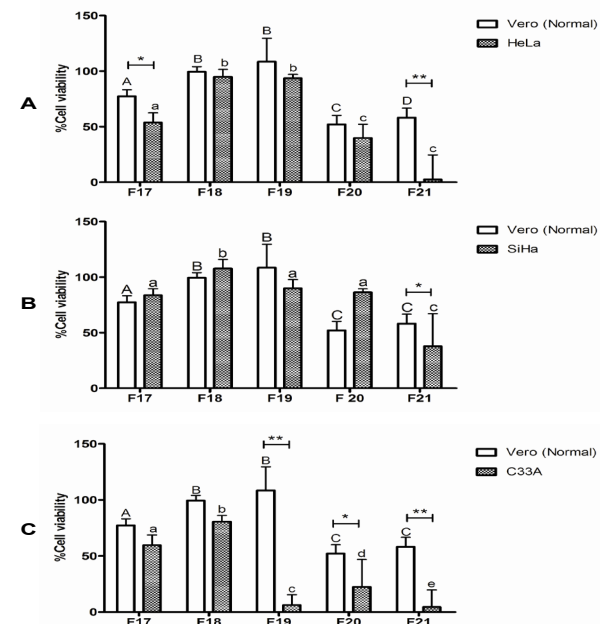


Figure 1. Cytotoxic effect of 24 hr treatment of 40 µg/ml F17-F21 on (A) HeLa (B) SiHa and (C) C33A cells compared to Vero cells. Values represent means±SD of six experiments in each group. The Mann-Whitney test was used. Values not sharing a common marking differ significantly at p<0.05. A, B, C and D indicate a significant difference among the fractions for cancer cells (p<0.05). a, b, c, and d indicate a significant difference among the fractions for Vero cells (p<0.05). A significant difference between cancer and Vero cells was indicated by *p<0.05 and **p<0.01

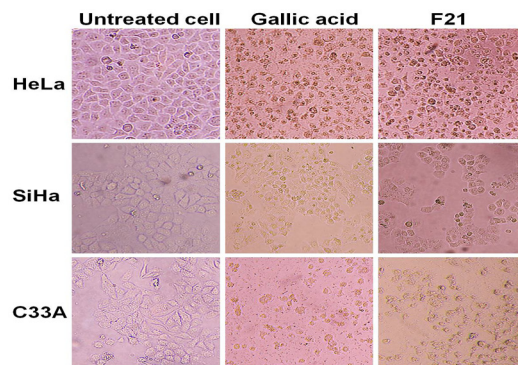


Figure 2. Morphological Alterations of HeLa, SiHa and C33A Cells Induced by Gallic Acid and F21. Cells were seeded in 75 mm plates and treated with F21 and gallic acid at 40 µg/ml for 24 hr. Photographs were taken directly from culture plates with a phase-microscope with a 100X objective

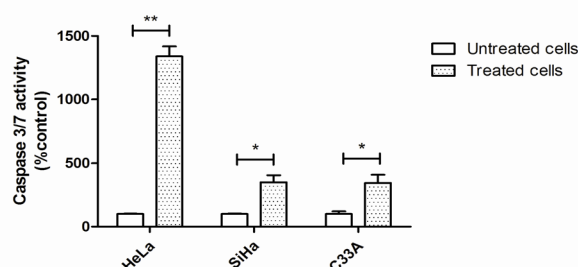


Figure 3. Caspase 3/7 Activation of Vero, HeLa, SiHa and C33A Cells Following Treatment with F21 at 40 µg/ml for 8 hr. Data represent mean±SD from six independent experiments *p<0.05 and **p<0.01 compared to the control

than gallic acid in all cells tested (Table 2). Phase-contrast micrographs demonstrated the morphologic alterations of F21- and gallic acid-treated cells (Figure 2).

Caspase assay measurement of apoptosis induction in cancer cells by F21

The caspase 3/7 assay (Figure 3) specifically identifies the activation of effector caspases 3 and 7. Treatment of HeLa, SiHa and C33A cervical cancer cell lines with F21 induced cellular morphological alterations consistent with initiated apoptosis. Thus, the caspase activity of HeLa, SiHa and C33A cells was examined. As shown in Figure 3, treatment with F21 caused a considerable increase in caspase activation in all cancer cells. At the same concentration of F21, HeLa cells had the highest caspase activity (6.5-fold) compared to the control.

Discussion

The young shoots and leaves of *C mimosoides Lamk* are used as a fresh dietary vegetable indicating possible safety for its future therapeutic utilization, and it has been reported to exhibit anti-microbial (Chanwitheesuk et al., 2007) and anti-oxidant activities (Chanwitheesuk et al., 2005). Potential anti-inflammatory diterpenoids have been isolated from the roots of *C mimosoides Lamk* (Yodsauoe et al., 2010). Moreover, the inhibitory effect of the methanolic crude extract from *C mimosoides Lamk*

against oral cavity cells but not normal cells is suggestive of preventive roles in cancer (Daduang et al., 2011). This study also demonstrated that the active fractions from *C mimosoides* Lamk enhanced killing of high-risk HPV-positive and HPV-negative cells.

This study showed that only the crude methanol extract (CME) of *C mimosoides* Lamk inhibited proliferation of cancer cell lines. Thus, CME was selected for further partial purification by silica gel column chromatography. From 27 fractions obtained, 5 fractions (F17-F21) exhibited an inhibitory effect against HeLa cell growth (Table 1). Similarly, previous phytochemical and bioactivity studies of *C mimosoides* Lamk have obtained bioactive substances from the polar components rather than the non-polar components (Chanwitheesuk et al., 2005; 2007; Kim, 2007; Yodsauoe et al., 2010).

The HPLC analysis results demonstrated that F17-F21 contained phenolic compounds, including gallic acid, caffeine, vanillic acid, ferulic acid, and resveratrol. Several studies revealed that these phenolic compounds have some biological properties, such as anti-oxidant, anti-microbial and anti-cancer activities (Chanwitheesuk et al., 2007; Kim, 2007; Frampton et al., 2010; Lu et al., 2010; Daduang et al., 2011; Hahnvajanawong et al., 2011; Niknafs, 2011; Sakong et al., 2011; Kim and Lee, 2012). Caffeine is thought to increase the anti-tumor effect of cisplatin or DNA-damaging agents because caffeine inhibits DNA repair. Caffeine-assisted chemotherapy has been used in the treatment of osteosarcomas (Kawano et al., 2012). Vanillic acid, a vegetable phenolic compound, is a strong antioxidant (Erdem et al., 2012). Moreover, ferulic acid enhances radiation effects by increasing lipid peroxidative markers in two cervical cancer cell lines, namely HeLa and ME-180, *in vitro* (Karthikeyan et al., 2011; Serafim et al., 2011). An *in vivo* model has shown that resveratrol has not only anti-inflammatory, anti-oxidant, anti-mutation, anti-proliferation and anti-cell cycle activities but also induces apoptosis and has synergistic effects with chemotherapeutic drugs (Frampton et al., 2010; Hahnvajanawong et al., 2011). F21 is composed of 68.49% gallic acid, and there are many studies that have reported the anti-cancer activity of this compound. Gallic acid can interfere with different stages of tumor development with the following activities: decreases the ornithine decarboxylase response linked to skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (Gali et al., 1991); inhibits P815 cell metastasis to the liver (Ohno et al., 2001); inhibits activator protein-1 (AP-1) transcriptional activity (Maggi-Capeyron et al., 2001); suppresses tumor angiogenesis (Liu et al., 2006b); inhibits the growth of HeLa cervical cancer cells via apoptosis and/or necrosis (You et al., 2010); decreases cell viability, proliferation, invasion and tube formation of gliomas (Lu et al., 2010) by suppressing ADAM17, which may contribute to the inhibition of invasiveness through the inactivation of PI3K/Akt and Ras/MAPK signaling pathways; and inhibits migration and invasion in human osteosarcoma U-2 OS cells through suppressing the matrix metalloproteinase-2/-9, protein kinase B (PKB) and PKC signaling pathways (Liao et al., 2012). In the present study, the effect of gallic acid alone and the F21 fraction was

investigated in human cervical cell lines (HeLa, SiHa, and C33A). HeLa and SiHa are cervical cancer cell lines that have been well characterized as both chemo- and radio-resistant (Saxena et al., 2005; Liu et al., 2006a). Therefore, these cells are suitable models to investigate the effect of phytochemicals. Differences in response to the agents were observed in the 3 cell lines. SiHa cells were the most resistant to gallic acid and F21. HeLa cells were the most sensitive to both gallic acid and F21 followed by C33A cells (Table 2). The IC₅₀ values of gallic acid from the current study were in close agreement with those reported in the literature (You et al., 2010). In addition, C33A cells were more sensitive to F19 treatments than the other cell lines, and F19 did not cause toxicity of normal cell lines. F19 contained caffeine and ferulic acid, which may indicate a synergistic effect. Thus, HPV-infected and non-HPV-infected cervical carcinoma treatment would be better served by different regimens.

C33A cells were the most drug sensitive, which was similar to a previous study on cellular response to chemotherapy and radiation in cervical cancer (Saxena et al., 2005). We demonstrated that HPV-16-containing cells were more resistant to phytochemical agents than HPV-18-containing cells and that the HPV-negative cell line (C33A) was the most sensitive. Interestingly, F21 killed more cells than gallic acid alone (Table 2). We found that the combination of phytochemical agents was affective in enhancing the cytotoxic effect in cervical cancer cells. Liu et al. (2006a) reported that both HeLa and SiHa cells are highly radioresistant and chemoresistant compared to other cervical cancer cell lines, but the present study indicated that the HeLa cell line was the most sensitive to multiple phytochemicals. However, it was also reported that the potency of cisplatin in combination with Noni were more in SiHa cells as compared to HeLa cells (Gupta et al., 2013).

During live cell imaging, cell morphology changes consistent with an apoptotic mechanism of cell death were observed. As a representative experiment, Figure 2 shows cancer cells treated with 40 µg/ml F21 and gallic acid for 24 hr. Features of cell shrinkage, irregularity in shape and cellular detachment were observed in these cells. The results obtained from the optical images were in good agreement with the NR assay results (Figure 1) where HeLa and C33A cells showed less than 20% viability when exposed to 40 µg/ml F21 and SiHa cells showed 60% viability under the same conditions.

To determine whether caspase activation was involved in F21-induced cell death, a caspase 3/7 assay was performed. As shown in Figure 3, treatment with 100 µg/ml F21 increased the caspase activity of HeLa, C33A and SiHa cells compared to the control (6.5-, 4- and 3-fold, respectively). Thus, F21 inhibited the growth of cervical cancer cells via apoptosis.

In conclusion, to our knowledge, this is the first report to study the inhibitory effect of multiple phenolic compounds from *C mimosoides* Lamk on HPV- and non-HPV-related cervical cancer cells. The susceptibility of all cancer cells was higher to F21 than gallic acid. F21 had a preferentially cytotoxic effect on cervical cancer cells compared to normal cells, and F21 also sensitized HeLa

cells to a higher degree than SiHa cervical cancer cells. The mechanism of the underlying effect of F21 may be the same as gallic acid, which induces cell death via apoptosis and/or necrosis (You et al., 2010). Therefore, F21 may be a potentially promising radiation-modifying drug and may improve therapeutic rates for the combination of F21 and ionizing radiation in the treatment of patients with cervical cancer. Further studies need to be performed to investigate if a multiple phytochemical combination approach is the most effective cancer treatment strategy to overcome drug toxicity and drug-induced resistance.

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