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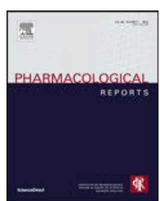

Original article

Cytotoxic effects of peanut phenolics possessing histone deacetylase inhibitory activity in breast and cervical cancer cell lines

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ABSTRACT

Background: Epigenetic histone modifications are considered as a promising avenue for cancer preventive and therapeutic strategies. The purpose of this study was to evaluate the antiproliferative and histone deacetylase (HDAC) inhibitory activity of selected peanut phenolics, including *p*-coumaric acid, ferulic acid, sinapinic acid and resveratrol, in MCF-7 and HeLa cells.
Methods: The cytotoxic and HDAC inhibitory activities were assessed by MTT assays, flow cytometric analyses of cell cycle arrest and apoptosis induction, and western blotting.
Results: The results showed that all four phenolics inhibited proliferation of both MCF-7 and HeLa cells in a dose-dependent manner. Among the phenolics tested, resveratrol was the most effective in inhibiting growth of cancer cells. Treatment with all phenolics resulted in histone H3 hyperacetylation in both cell lines, indicating potential for HDAC inhibition. These phenolics induced apoptosis in both MCF-7 and HeLa cells in a concentration-dependent manner. Moreover, all phenolics induced G0/G1-phase arrest of the cell cycle in MCF-7 cells while *p*-coumaric and ferulic acids caused S-phase arrest in HeLa cells. Exposure to *p*-coumaric acid increased p53 and p21 expression but decreased CDK4 levels in both cell types, which could result in the observed G0/G1 arrest. Moreover, inhibition of ERK1/2 phosphorylation by ferulic acid and resveratrol contributed to cell growth inhibition.
Conclusion: Peanut phenolics appear to influence the extent of histone acetylation in MCF-7 and HeLa cells, and this activity modulates multiple pathways that are implicated in cancer prevention.
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Introduction

Cancer development and progress results from genetic mutations and epigenetic alterations [1]. Aberrant patterns of histone modifications are associated with carcinogenesis [2]. The acetylation/deacetylation of histones, catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, directly impacts gene expression in both transformed and untransformed cells. Aberrant expression and function of HDACs are involved in loss of protein expression responsible for growth inhibition and induction of apoptosis, which lead to uncontrolled growth of cancer cells [2,3].

HDAC inhibitors are key agents for restoring expression of the tumor suppressor genes and repressing proto-oncogenes [4]. HDAC inhibitors have been reported to turn on tumor suppressor genes p53 [5] and p21$^{(WAF1)}$ [6]. Growing evidence suggest that HDAC inhibitors are capable of arresting cell growth, inducing differentiation and/or apoptosis of a number of cancer cell lines [7]. Recently, dietary phytochemicals possessing HDAC inhibitory activity were shown to trigger DNA damage and repair mechanisms in cancer cells [8]. The ATM checkpoint pathway leads to a set of diverse DNA damage responses including the activation of

Abbreviations: ATM, ataxia telangiectasia mutated; CDK, cyclin-dependent kinase; DMSO, dimethyl sulfoxide; HAT, histone acetyltransferase; HDAC, histone deacetylase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NaB, sodium butyrate; PI, propidium iodide; PVDF, polyvinylidene fluoride; RNase A, ribonuclease A.

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signal transduction, cell cycle regulation, DNA repair and apoptosis [9]. It has been shown that increasing ATM signaling and p53 phosphorylation lead to p21 induction and G0/G1 arrest in breast cancer cells [10]. A growing number of clinical trials using phenolic compounds and polyphenol-rich extracts in chemoprevention and therapy suggest that such compounds may serve as valuable alternatives to chemotherapeutics [11]. These compounds and their congeners act pleiotropically on cancer cells to induce cell-cycle arrest, and exert anti-proliferation, pro-apoptotic and anti-angiogenic activities [12,13].

Breast cancer is the leading cause of cancer death among females worldwide, while cervical cancer is the fourth leading cause. About 50% of breast cancer and nearly 90% of cervical cancer cases are found in developing countries of the world [14,15]. While many cancer patients initially respond to chemotherapy, resistance often ensues which leads to poor prognosis. In addition, adverse side effects and toxicities limit the clinical use of chemotherapy, which underscores the urgent need for novel drug with improved efficacy and less deleterious effects.

Fruits, vegetables and peanuts are major sources of phenolic compounds. Some phenolic-rich extracts of peanut testae have been demonstrated to possess HDAC inhibitory and anticancer activities against human cancer cells [16]. Interestingly, phenolics suchas resveratrol, *p*-coumaric, ferulic, and sinapinic acids have been found in peanuts [17,18]. The objectives of this study were to assess cytotoxic effect and HDAC inhibitory activity of peanut phenolics in MCF-7 and HeLa cells and to elucidate the underlying mechanisms. This study provides insight into the role of phenolics in the possible mechanisms for cancer prevention and cure.

Materials and methods

Cell culture

Human breast adenocarcinoma (MCF-7 cells), human cervical adenocarcinoma (HeLa cells) and non-cancer (Vero cells; African green monkey kidney epithelial cells) cell lines were obtained from Dr. O. Tetsu (University of California, San Francisco, USA), Dr. P. Picha (National Cancer Institute, Bangkok, Thailand) and Dr. S. Barusrux (Khon Kaen University, Thailand), respectively. All cell lines were maintained in RPMI-1640 medium (Gibco-BRL) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% heat-inactivated fetal bovine serum. The cells were incubated with an atmosphere of 5% CO_2 at 37 °C. Propidium iodide (PI), sodium butyrate (NaB; purity ≥ 98.5%), resveratrol (purity ≥ 99.0%), *p*-coumaric (purity ≥ 98.8%), ferulic (purity ≥ 99.0%) and sinapinic acids (purity ≥ 98.0%) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). The antibodies including anti-Ac-H3, anti-p21, anti-CDK4, anti-p53, anti-Bcl-2, anti-pERK1/2 (phospho-Thr202/Tyr204), anti-ERK1/2, and anti-β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA). Annexin V-FITC was purchased from Biolegend (San Diego, CA, USA). The MTT was purchased from Invitrogen, Molecular Probes products (Eugene, Oregon, USA).

Cell proliferation assays

Cell proliferation was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MCF-7, HeLa and Vero cells were seeded in 96-well plates at a density of 8×10^3 cells/well and incubated for 24 h. Cells were treated with increasing concentrations of resveratrol, ferulic, *p*-coumaric and sinapinic acids for 24, 48 and 72 h. DMSO (0.5%) was used as a solvent control. After indicated time the culture medium was replaced with fresh media containing 1.2 mM MTT and incubated for 2 h at 37 °C. DMSO (100 μL) was added to each well to dissolve the formazan formed due to MTT reduction. Absorbance of formazan was measured with a Spectramax M5 microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) at 550 nm with a reference wavelength of 655 nm. Cell viability was then calculated by plotting curve and expressed as percentage.

Apoptosis analysis by flow cytometry

Apoptosis was assessed with flow cytometric analysis of cells using Annexin V-FITC and PI staining (Annexin V-FITC, BioLegend, San Diego, CA) according to the manufacturer's instruction. Briefly, MCF-7 and HeLa cells were seeded (1×10^6 cells) into a 5.5-cm dish and incubated for 24 h. After that MCF-7 cells were treated with different concentrations of resveratrol (50, 100, 150 μM), *p*-coumaric (3–5 mM), ferulic (3–5 mM), and sinapinic acids (3–5 mM) or vehicle (0.3% DMSO) for 48 h. HeLa cells were treated with resveratrol (50, 100, 150 μM), *p*-coumaric (1–3 mM), ferulic (1, 2 and 3 mM), and sinapinic acids (1, 2 and 3 mM) for 24 h. After indicated period, cells were harvested, centrifuged and washed twice with ice-cold PBS. Cells were suspended in 100 μL ice-cold Annexin-binding buffer and subsequently stained with Annexin V-FITC and PI. The stained samples were kept in dark for 15 min at room temperature and then analyzed by BD FACSCantoII Flow Cytometer (Becton Dickinson, San Jose, CA, USA) (The service was provided by Research Instrument Center, Khon Kaen University, Thailand).

Cell cycle analysis by flow cytometry

Cell cycle phase distribution was evaluated using PI staining. Cells were seeded on cell culture dish (1×10^6 cells/dish) for 24 h and treated with peanut phenolics for 48 h. After that, the cells were harvested, centrifuged and fixed in ice cold 70% ethanol. Fixed cells were re-suspended in 300 μL of PBS. The samples were incubated with RNaseA (final concentration 0.2 mg/mL) at 37 °C for 1 h. Cells were stained with PI solution (final concentration of 50 μg/mL) at room temperature for 45 min. Labelled cells were analysed by flow cytometry as described above.

Western blot analysis

Cells were seeded on cell culture dish (1×10^6 cells/dish) for 24 h and treated as mentioned above in apoptosis analysis. Sodium butyrate (NaB), a well known HDAC inhibitor was used for comparative purposes. Protein expression levels of interest were detected by western blotting detection method. Total cellular proteins were extracted from drug-treated and solvent-treated cells using RIPA lysis buffer. Equal amounts of protein (30 μg) were separated on 10-12.5% SDS-PAGE and transferred to a PVDF membrane. The membranes were then blocked with 5% skim milk for 1 h, and subsequently incubated overnight with primary antibodies: anti-p21, -acetylated H3, -CDK4, -pERK1/2, -ERK1/2, -p53 or -β-actin. Proteins were probed with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature. Protein signal bands were developed by using ECL Prime Chemiluminescence (Amersham).

Statistical analysis

Data are expressed as means ± standard deviation (SD) from three independent experiments. Duncan's *post hoc* test was carried out using statistical program of IBM SPSS Statistic version 20.0 for mac (SPSS Corporation, Chicago, IL, USA). Significant differences ($p < 0.05$) among phenolics were analyzed by using one-way ANOVA.

Table 1
Antiproliferative effects of four phenolics on MCF-7 and HeLa cells.

Phenolic compounds	IC_{50} Values*								
	MCF-7 cells			HeLa cells			Vero cells		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
p-Coumaric acid (mM)	>5	4.77 ± 0.21	4.47 ± 0.11	2.16 ± 0.12	1.15 ± 0.10	0.94 ± 0.04	>5	3.74 ± 0.30	2.89 ± 0.10
Ferulic acid (mM)	4.07 ± 0.03	4.09 ± 0.02	3.97 ± 0.12	2.56 ± 0.03	1.87 ± 0.14	0.88 ± 0.14	>5	3.45 ± 0.61	2.80 ± 0.06
Sinapinic acid (mM)	>5	4.33 ± 0.13	4.02 ± 0.05	2.58 ± 0.26	1.76 ± 0.23	1.75 ± 0.03	>5	>5	>5
Resveratrol (μM)	101.85 ± 2.93	67.32 ± 3.76	46.81 ± 1.26	66.33 ± 8.06	62.48 ± 0.66	53.02 ± 2.42	>200	199.55 ± 6.71	167.67 ± 0.77
Sodium butyrate** (mM)	>5	>5	>5	>5	3.60 ± 0.16	1.78 ± 0.17	>5	4.65 ± 0.65	3.85 ± 0.34

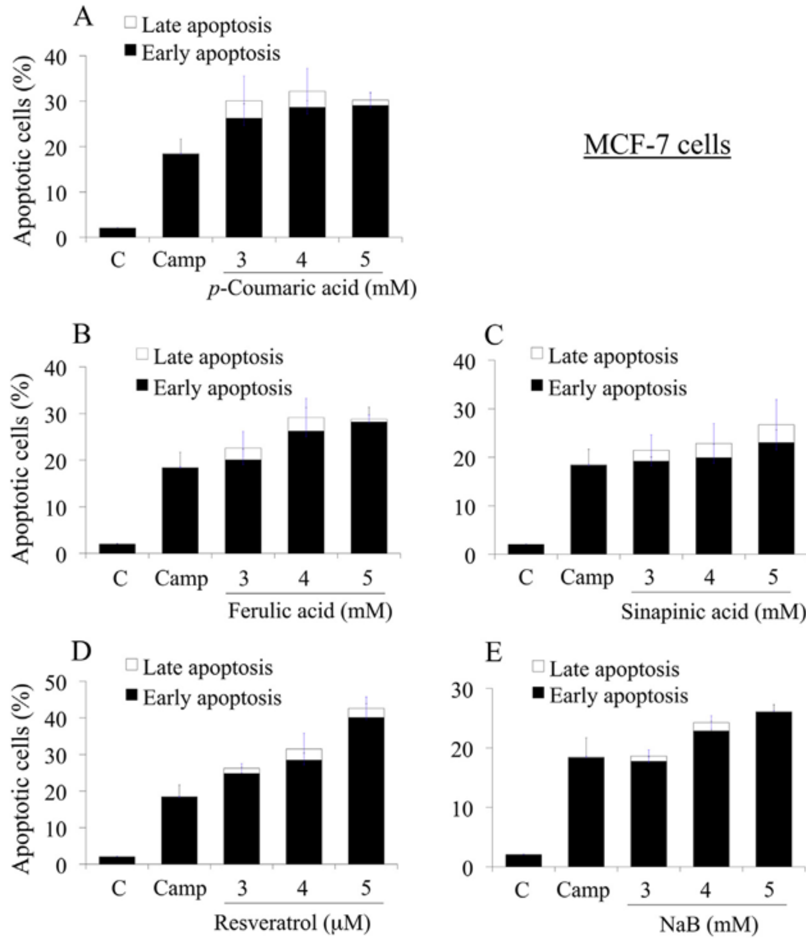

Fig. 1. Flow cytometric analysis of apoptosis induction in MCF-7 cells. Cells were treated with various concentrations of *p*-coumaric acid (A), ferulic acid (B), sinaipinic acid (C), resveratrol (D) and NaB (E) for 48 h. The appearance of apoptotic cells was analyzed by flow cytometry using Annexin V-FITC/PI staining. Camptothecin (Camp; 25 μM) was used as a positive control. Bar graph shows the summarized data from three independent experiments performed in duplicate compared with untreated control (C).

Results

Growth inhibition of selected peanut phenolics in MCF-7 and HeLa cells

Our previous study [16] demonstrated that peanut testa extracts inhibited growth of MCF-7 and HeLa cells. To determine the cytotoxic effect of peanut phenolics that may underpin cytotoxic effect of peanut testa extracts, four selected peanut phenolics, *p*-coumaric acid, ferulic acid, sinapinic acid and resveratrol, were investigated for their cytotoxic effects in both MCF-7 and HeLa cells. The IC_{50} values (concentrations required to decrease cell viability by 50%) were determined and compared with the HDAC inhibitor sodium butyrate (NaB). The IC_{50} values shown in Table 1 indicated that resveratrol significantly inhibited proliferation of MCF-7 and HeLa cells, with IC_{50} values of 46.81 ± 1.26 and 53.02 ± 2.42 μM, respectively, for 72 h treatments. Other peanut phenolics also inhibited proliferation of both cancer cell lines, but with higher IC_{50} values. However, all four peanut phenolics inhibited cell proliferation more effectively than NaB. In addition, non-cancer (Vero) cells were more resistant to the growth inhibitory effects of phenolic treatments than either MCF-7 or HeLa cells (Table 1).

Effect of selected peanut phenolics on apoptosis induction in MCF-7 and HeLa cells

The apoptosis-inducing ability of peanut phenolics in MCF-7 and HeLa cell lines was assessed by Annexin V and PI staining. The results shown in Figs. 1 and 2 revealed that resveratrol significantly induced apoptosis of both MCF-7 and HeLa cells in a dose-dependent manner, with a higher percentage of apoptotic cells compared with other phenolics. Sinapinic acid was less effective than NaB for inducing apoptosis in MCF-7 cells (Fig. 1). However, all peanut phenolics were more effective than NaB for inducing apoptosis in HeLa cells (Fig. 2).

Effect of selected peanut phenolics on cell cycle progression in MCF-7 and HeLa cells

To evaluate the effect of peanut phenolics on cell cycle progression, we analyzed cell cycle profiles of PI-stained cells by flow cytometry. Comparing to untreated control (Fig. 3A), all four phenolics and NaB caused cell cycle arrest at G0/G1 phase in MCF-7 cells (Figs. 3B-3P). Exposure to *p*-coumaric acid (3 mM), ferulic acid (3 mM), sinapinic acid (3 mM) and resveratrol (100 μM), caused G0/G1 arrest with $65.0 \pm 3.6\%$ (Fig. 3B), $56.9 \pm 2.0\%$ (Fig. 3E),

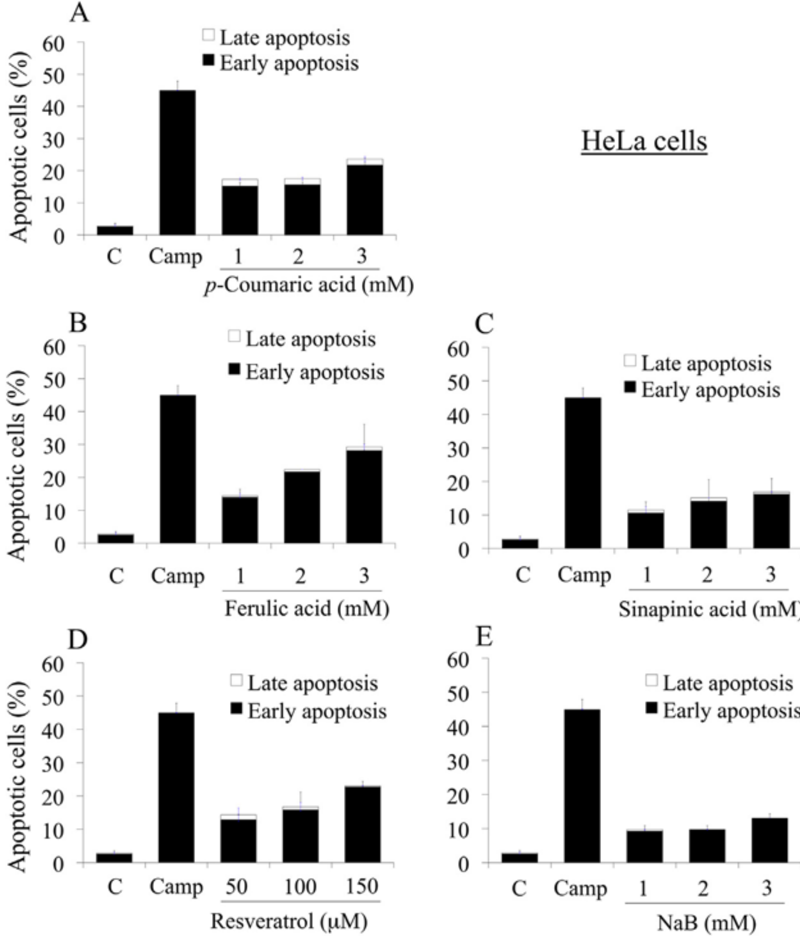

Fig. 2. Flow cytometric analysis of apoptosis induction in HeLa cells. Cells were treated with various concentrations of *p*-coumaric acid (A), ferulic acid (B), sinaipinic acid (C), resveratrol (D) and NaB (E) for 24 h. The appearance of apoptotic cells was analyzed by flow cytometry using Annexin V-FITC/PI staining. Camptothecin (Camp; 25 μM) was used as a positive control. Bar graph shows the summarized data from three independent experiments performed in duplicate compared with untreated control (C).

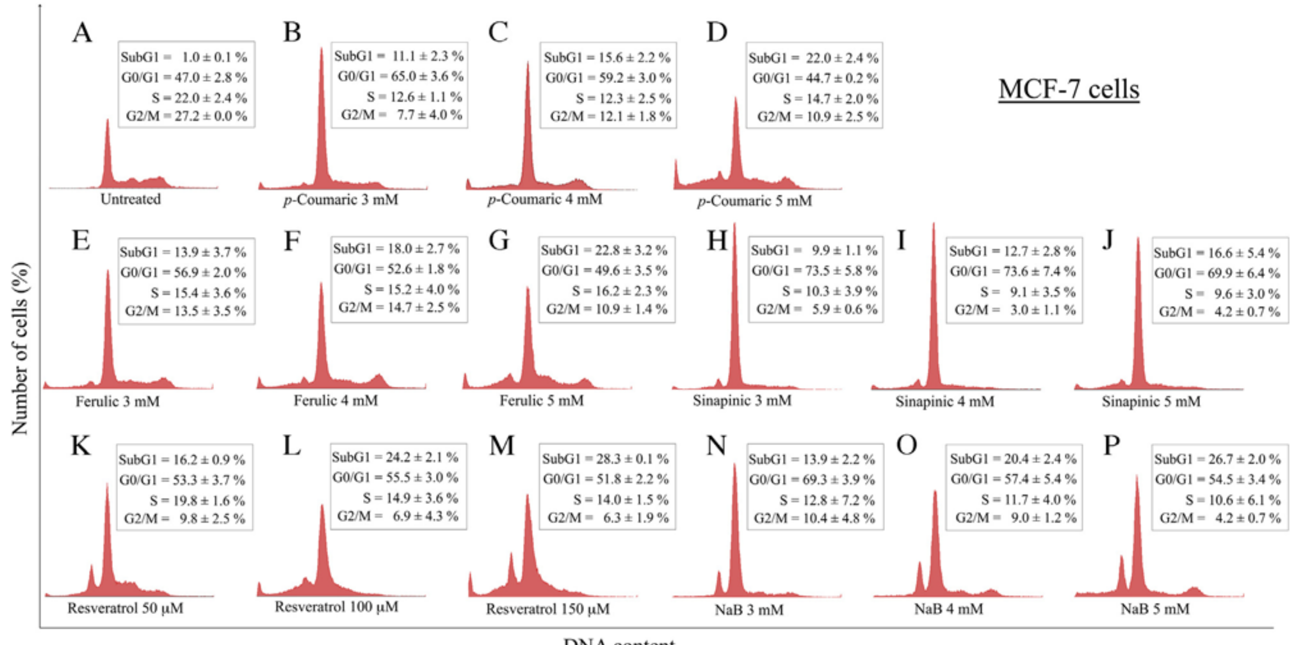

Fig. 3. Flow cytometric analysis of cell cycle arrest in MCF-7 cells. Cells were treated with various concentrations of *p*-coumaric acid (B–D), ferulic acid (E–G), sinaipinic acid (H–J), resveratrol (K–M) and NaB (N–P) for 48 h. After harvesting, cells were labeled with PI and analyzed by flow cytometry. The histograms show the percentage of cells in each phase of the cell cycle. Results were obtained from three independent experiments and presented as means ± SD.

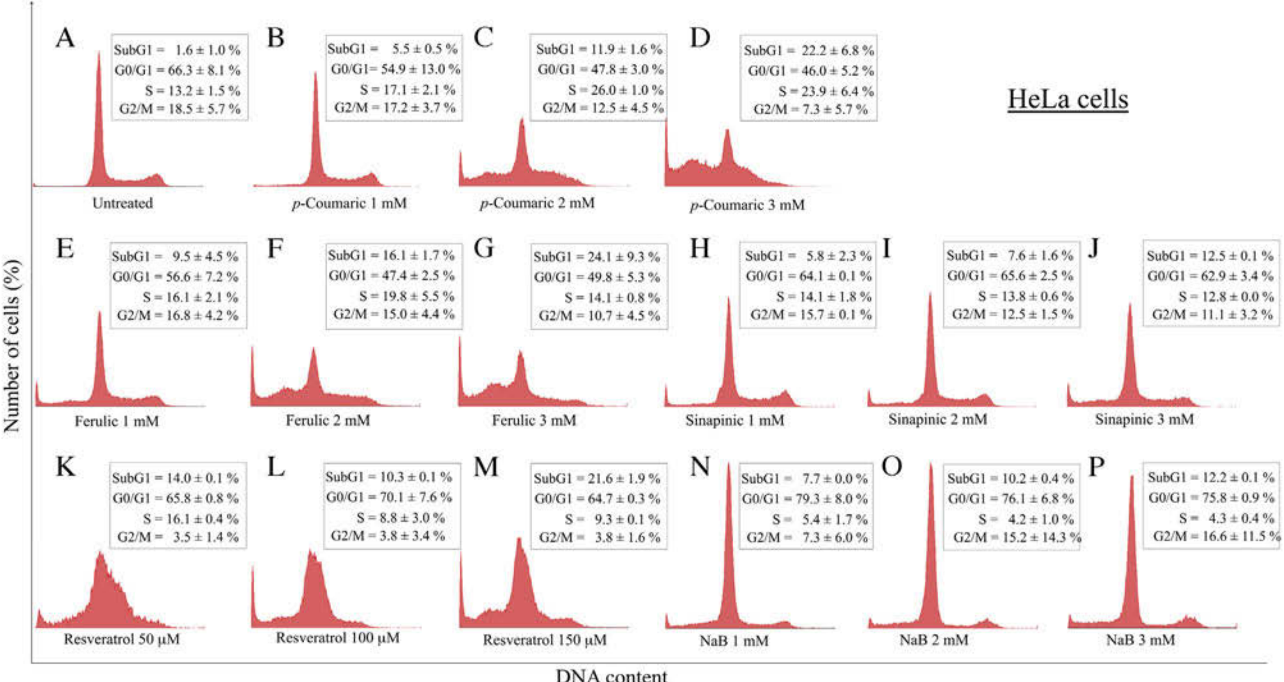

Fig. 4. Flow cytometric analysis of cell cycle arrest in HeLa cells. Cells were treated with various concentrations of *p*-coumaric acid (B–D), ferulic acid (E–G), sinaipinic acid (H–J), resveratrol (K–M) and NaB (N–P) for 24 h. After harvesting, cells were labeled with PI and analyzed by flow cytometry. The histograms show the percentage of cells in each phase of the cell cycle. Results were obtained from three independent experiments and presented as means ± SD.

73.5 ± 5.8% (Fig. 3H), and 55.5 ± 3.0% (Fig. 3L) of cells, respectively. A corresponding reduction in the cell population was found in S and G2/M phases. In addition, increased sub-G1 fraction was observed with higher concentration of all phenolics, indicating more cell death.

In HeLa cells (Fig. 4A–P), some phenolic treatments caused cell cycle arrest in S phase. Compared to the untreated control (13.2 ± 1.5%) (Fig. 4A), *p*-coumaric acid (2 mM) caused the greatest accumulation of cells in S phase (26.0 ± 1.0%) (Fig. 4C). Ferulic acid (2 mM) also caused a high accumulation of cells in S phase (19.8 ± 5.5%) (Fig. 4F). In addition, treatments with increasing concentrations of some phenolic compounds caused cell death, as evidenced by more cells in the sub-G1 population. For example, sinapinic acid (Fig. 4H–J) did not induce cell cycle arrest at the concentrations tested, but induced sub-G1 arrest cell death at higher concentrations.

Effect of peanut phenolics on histone H3 acetylation and modulation of cell cycle, apoptosis and proliferation

Because our previous study [16] provided evidence of HDAC inhibition by peanut testa extracts, we conducted western blotting to investigate the level of histone H3 acetylation in MCF-7 and HeLa cells before and after treatment with the four peanut phenolics. All four phenolics increased histone H3 acetylation in a concentration-dependent fashion (Figs. 5 and 6). Resveratrol exhibited the most potent HDAC inhibitory activity in regard to histone H3 hyperacetylated proteins in both MCF-7 and HeLa cells.

Since the above results provided evidence of apoptosis induction (Figs. 1 and 2) and cell cycle arrest (Figs. 3 and 4) by peanut phenolics, we therefore assessed protein factors associated with cell-cycle arrest and apoptosis (p53, p21, CDK4, Bcl-2) in MCF-7 and HeLa cells as presented in Figs. 5 and 6, respectively. Treatments with *p*-coumaric acid (Figs. 5 A and 6 A), ferulic acid (Figs. 5 B and 6 B) and resveratrol (Figs. 5D and 6D) led to an increase in p53 protein levels in both MCF-7 and HeLa cells. However, sinapinic acid (Figs. 5 C and 6 C) did not induce any variations in p53 expression in either cell line at concentrations tested. All four phenolics exerted variable effects on expression of p21 in MCF-7 and HeLa cells. Ferulic acid, sinapinic acid and resveratrol all decreased p21 expression level in MCF-7 cells. However, *p*-coumaric acid caused an increase in p21 expression in both MCF-7 and HeLa cells. The expression of CDK4 was decreased in a dose-dependent manner after treatment with all phenolics in both MCF-7 and HeLa cells. Bcl-2 expression in HeLa cells was down-regulated by treatment with ferulic acid, sinapinic acid and resveratrol. However, expression of Bcl-2 was not altered in MCF-7 cells by any of these compounds.

MAPK/ERK activation is associated with cell survival, however, DNA damage-induced activation of ERK1/2 results in cell cycle arrest and apoptosis [33]. Since the effect of peanut phenolics on ERK1/2 signaling pathway was limited, we determined the level of pERK upon treatment with peanut phenolics in both MCF-7 and HeLa cells. As presented in Figs. 5 and 6, ferulic acid and resveratrol down-regulated the levels of pERK1/2 in both MCF-7 and HeLa cells while *p*-coumaric acid had the opposite effect. Finally, sinapinic acid treatments increased pERK1/2 levels in MCF-7 cells, but decreased pERK1/2 levels in HeLa cells.

Discussion

Natural phytochemicals possess great potential for reversing adverse epigenetic events related with carcinogenesis [19]. Previously, we showed that peanut phenolic extracts inhibited cancer cell proliferation *via* induction of apoptosis along with HDAC inhibition [16]. In this study, we demonstrated that peanut phenolics including *p*-coumaric acid, ferulic acid, sinapinic acid and resveratrol, exerted dose-dependent, anti-proliferative effect

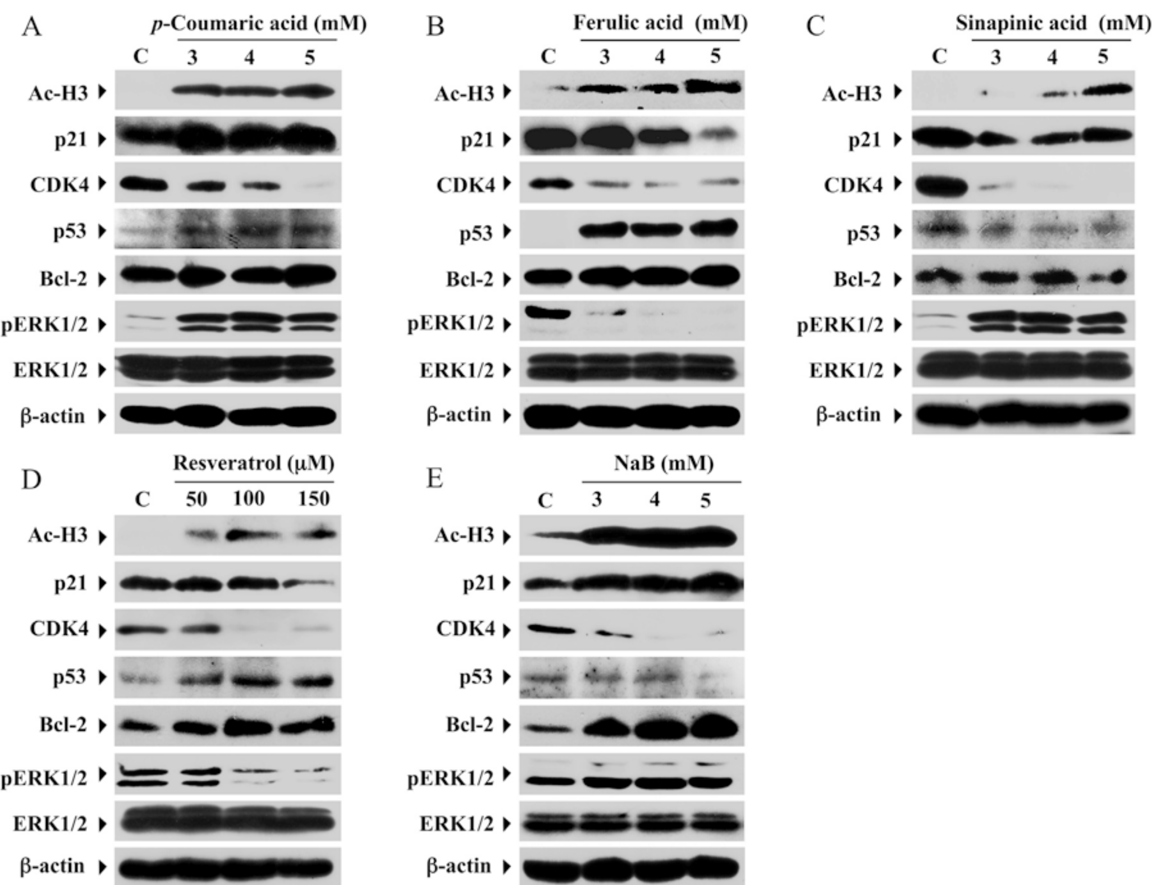

Fig. 5. Western blot analysis of acetylated histone H3, p53, p21, CDK4, Bcl-2 and pERK1/2 expression in MCF-7 cells. Cells were treated with various concentrations of *p*-coumaric acid (A), ferulic acid (B), sinaipinic acid (C), resveratrol (D) and NaB (E) for 48 h. Total proteins were extracted, separated on SDS-PAGE, and transferred onto PVDF membrane. Specific proteins were detected using the appropriate antibodies.

on MCF-7 and HeLa cells, which may result from cell cycle arrest and induction of apoptosis. In addition, we investigated HDAC inhibitory activity of all four compounds in both cancer cell lines.

HDAC inhibitors are considered to be therapeutic candidates for cancer by virtue of their ability to enhance expression of tumor suppressor genes, repress expression of oncogenes, and induce apoptosis through generation of ROS and DNA damage [4,20]. The CDK inhibitor p21 is one of the most commonly induced genes by HDAC inhibitors. HDAC inhibitors induced p21 expression in p53-dependent and –independent pathways, which correlates with an increase in the acetylation of histones at p21 promoter region [21,22]. Consistently, *p*-coumaric acid and NaB, both HDAC inhibitors, increased p21 level in MCF-7 and HeLa cells (Figs. 5 A,E, 6 A,E). However, p21 levels were decreased by treatment of MCF-7 and HeLa cells with other phenolic compounds (Figs. 5B–D and 6 C,D). Genomic mutation is a key feature of carcinogenesis that enables tumor cells escape from cell cycle checkpoint controls and apoptosis. Tumor cells may accumulate many genomic defects that contribute to DNA-damage response leading to cell death [23,24]. HDAC inhibitors alter the state of histone modifications and activate the ATM-dependent, DNA damage checkpoint pathway that is involved in transcriptional regulation of cancer related genes [25]. The resultant DNA damage can activate apoptosis and/or cell cycle arrest, both of which may contribute to growth inhibition.

Our results suggest that *p*-coumaric acid, ferulic acid, sinapinic acid and resveratrol found in peanut testae altered the extent of histone acetylation in MCF-7 and HeLa cells in a manner that was similar to the well known HDAC inhibitor sodium butyrate (Figs. 5 and 6). Valproic acid, another well-known HDAC inhibitor, also exhibited HDAC inhibitory and anti-proliferative activities in both MCF-7 [26] and HeLa [27] cells.

Many natural products act as HDAC inhibitors and anti-proliferative agents in tumor cells. For example, chrysin, an HDAC inhibitor of plant origin, induces p21 expression and cell cycle arrest, reportedly by increasing phosphorylation of p53 [28]. Similarly, psammaplin A, a natural phenolic with HDAC inhibitory activity, induced $p21^{(WAF1)}$ expression, cell cycle arrest, and apoptosis in p53-independent manner [29]. These effects were very similar to those of *p*-coumaric and ferulic acids in MCF-7 and HeLa cells, respectively, reported herein. However, the peanut phenolics studied herein appeared to exert cell-specific activity. For example, resveratrol induced up-regulation of p53 and down-regulation of p21 in both MCF-7 and HeLa cells (Figs. 5 D and 6 D), and ferulic acid had similar effects in MCF-7 cells (Fig. 5D). This may be due to activation of the p53 pathway upon DNA damage. In addition, down-regulation of p21, in some contexts, is important for induction of DNA damage-mediated apoptosis [30]. In this study, apoptosis induced by peanut phenolics was evidenced by Annexin V-FITC staining.

We observed that treatments with ferulic acid, sinapinic acid and resveratrol decreased the level of anti-apoptotic Bcl-2 protein in HeLa cells (Fig. 6B–D) in a manner similar to that of the phenolic curcumin in glioma cells [31]. However, treatment with most of the

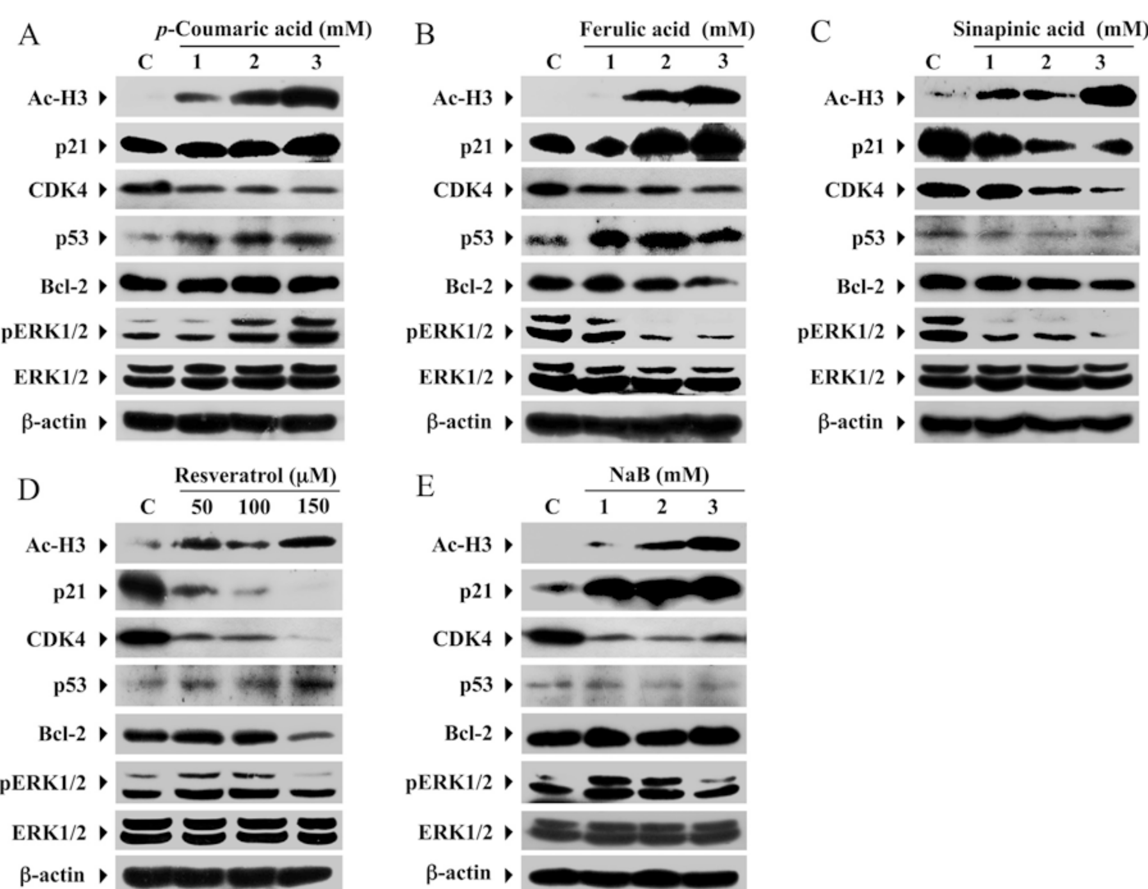

Fig. 6. Western blot analysis of acetylated histone H3, p53, p21, CDK4, Bcl-2 and pERK1/2 expression in HeLa cells. Cells were treated with various concentrations of *p*-coumaric acid (A), ferulic acid (B), sinaipinic acid (C), resveratrol (D) and NaB (E) for 24 h. Total proteins were extracted, separated on SDS-PAGE, and transferred onto PVDF membrane. Specific proteins were detected using the appropriate antibodies.

phenolic compounds studied herein, except sinapinic acid, increased expression of Bcl-2 protein in MCF-7 cells. Increasing levels of Bcl-2 are often associated with escape of apoptosis by cancer cells [32]. These results suggest that the effect of phenolics on Bcl-2 expression and cell cycle was complex. It can be speculated that several parallel pathways may be involved in the regulation of apoptosis in both HeLa and MCF-7 cells. First, all the phenolics studied caused G1-phase arrest in MCF-7 cells but only resveratrol induced G1-phase arrest in HeLa cells. Blockage of cell cycle progression of MCF-7 and HeLa cells by phenolics was most likely due to suppression of CDK4 expression (Figs. 5 and 6). Consistently, the plant HDAC inhibitor lycorine induced G0/G1 arrest in human leukemic cell line K562 through down-regulation of CDK4 and cyclin D1 expression [33]. Resveratrol has previously been shown to inhibit the growth of many types of human cancer cells *via* cell cycle arrest, apoptosis induction and modulation of biomarker expression [34–36]. However, the anti-proliferative effect of resveratrol may also involve regulation of the activity of SIRT1, a NAD^+-dependent deacetylase regulating metabolism and aging processes [37].

Although activation of ERK1/2 generally promotes cell survival, under certain conditions, ERK1/2 activation can also exert pro-apoptotic functions [38]. In this study, some phenolic treatments caused both ERK1/2 activation and apoptosis induction as supported by flow cytometry-based apoptosis detection. In agreement with our finding, triptolide induced ERK activation and subsequent caspase-dependent cell death in relation to ROS generation and ER stress [39]. It was reported that triptolide treatment changed Bcl-2 protein expression *via* ERK activation. In addition, etoposide induced ERK1/2 activation in MCF7 cells, which led to both cell cycle arrest and apoptosis [40]. In contrast, some phenolic treatments promoted both inhibition of ERK1/2 signaling (Figs. 5 B,D and 6 B–D) and apoptosis induction (Figs. 1 B,D and 2 B–D). Consistently, natural compounds rocaglamides inhibited ERK1/2 activation leading to apoptosis or cell cycle arrest [41].

Based on our findings, it can be concluded that cancer preventive effect of selected peanut phenolics are attributed to induction of apoptosis and cell cycle arrest mediated, at least in part, by core histone modification. The antiproliferation properties of these phenolics with HDAC inhibitory activity have been associated with modulation of various apoptotic and/or cell cycle related proteins, including Bcl-2, p53, p21, CDK4 and pERK1/2. Our findings provide fundamental information for further studies on phenolics alone or in combination with other therapeutic modalities, and suggest phenolics as chemopreventive and therapeutic candidates for breast and cervical cancers. In this regard, nutritional epigenetics may be viewed as an attractive tool to prevent carcinogenesis.

Conflict of interest

None.

Acknowledgements

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