Ellagic Acid, a Dietary Polyphenol, Selectively Cytotoxic to HSC-2 Oral Carcinoma Cells

JEFFREY H. WEISBURG, ALYSSA G. SCHUCK, SARAH E. REISS, BELLA J. WOLF, SIMONE R. FERTEL, HARRIET L. ZUCKERBRAUN and HARVEY BABICH

Department of Biology, Stern College for Women, Yeshiva University, New York, NY, U.S.A.

Abstract. *Background: The antiproliferative and apoptotic effects of ellagic acid, a dietary polyphenol, were studied. Materials and Methods: The neutral red cytotoxicity assay compared the sensitivities of gingival fibroblasts and HSC-2 oral carcinoma cells to ellagic acid. The ferrous ion oxidation xylenol orange assay and levels of intracellular reduced glutathione were used to assess pro-oxidant nature of ellagic acid. Antioxidant activity was demonstrated in cells co-treated with H_2O_2 and ellagic acid by 2',7'-dichlorodihydrofluorescein diacetate staining and in cells co-treated with gallic acid and ellagic acid by morphological analysis. Apoptosis was assessed by microscopy, flow cytometry, luminescence, and immunoblotting. Results: Ellagic acid was cytotoxic to carcinoma cells, but not to normal cells. Its pro-oxidant nature was minimal, whereas its antioxidant property was biologically significant. Ellagic acid-treated cells demonstrated apoptotic morphology, induction of apoptosis (flow cytometry), increase in caspase 3/7 activities (luminescence), and activation of caspase 3 and cleavage of poly ADP ribose polymerase (immunoblot). Conclusion: Ellagic acid exhibited significant antioxidant, but not pro-oxidant, activity and was selectively cytotoxic to oral carcinoma cells.*

Epidemiological studies have indicated that diets rich in fruits, vegetables, and nuts promote health and well-being and reduce the risk of various chronic pathologies. Research in cancer prevention, in particular, has benefited tremendously from the discovery that many natural plant products exert anticancer effects. Many of these studies, conducted with human cells *in vitro*, have evaluated plant extracts or specific phytochemicals for their cancer chemopreventive properties. Plant-based food products contain high levels of a diverse spectrum of phytochemicals, of which the polyphenols are the most significant. This research evaluated the anticancer effects of ellagic acid (Figure 1), common in green tea, grape seed extract, pomegranate, strawberries, blackberries, raspberries, and walnuts. The focus of this research was the oral cavity, as oral cancer is one of the most disfiguring types of cancer, since surgical removal of the tumor may lead to facial disfigurement, and as oral squamous cell carcinoma is the fifth most common type of cancer worldwide (1).

Polyphenols are interesting in that they exhibit both antioxidant and pro-oxidant activities (2), either or both of which may be significant enough to exert biological effects. For example, the cytotoxicity of epigallocatechin gallate, the main polyphenol in green tea, and of theaflavin-3,3'-digallate, the main polyphenol in black tea, correlated with their strong pro-oxidant property (3, 4). Conversely, epicatechin gallate, a green tea polyphenol and a poor generator of oxidants, exerted cytotoxicity that was independent of its pro-oxidant property (5). Although the antioxidant nature of ellagic acid has been noted (6), there is little information on its prooxidant nature and whether it is sufficient to evoke a biologic response from target cells. Using HSC-2 oral carcinoma cells, the pro-oxidant and antioxidant properties of ellagic acid were studied.

In vitro research with ellagic acid has shown its ability to exert growth-inhibiting and apoptosis-inducing cytotoxicity towards cancer cells, including pancreatic (7), breast, prostate gland (8), leukemic (9), neuroblastoma (10), colon (11), osteogenic sarcoma (12), and tongue (13) cells. As far as we know, there are no studies on the comparative responses of human oral cavity carcinoma cells and normal gingival fibroblasts to ellagic acid. The studies presented herein compared the antiproliferative activity of ellagic acid on HSC-2 carcinoma cells isolated from the oral palate to that on normal gingival fibroblasts.

Correspondence to: Dr. J.H. Weisburg, Department of Biology, Stern College for Women, 245 Lexington Avenue, New York, NY 10016 U.S.A. Tel: +1 2123407869, Fax: +1 2123407883, e-mail: weisburg@yu.edu

Key Words: Ellagic acid, pro-oxidant, antioxidant, antiproliferative, apoptosis, H5C-2 oral carcinoma cells.

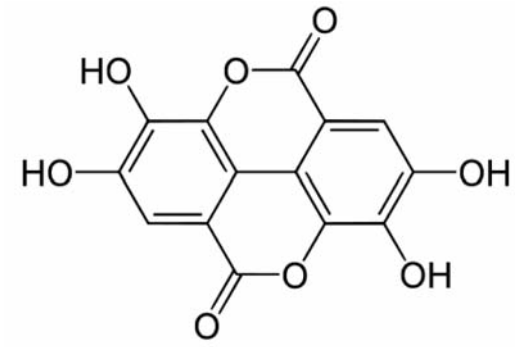

Figure 1. *Molecular structure of ellagic acid.*

Materials and Methods

Cells. HSC-2 Human squamous carcinoma cells derived from the oral cavity were obtained from H. Sakagami, Department of Dental Pharmacy, Meikai University School of Dentistry. Saitama, Japan, and normal human HF-1 fibroblasts from P. Sacks, New York University School of Dentistry, New York, NY, USA. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 110 mg/l pyruvate (cat. no. 11995-080), supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin G, 50 μg/ml streptomycin sulfate, and 1.25 μg/ml amphotericin B, termed the growth medium, and were maintained in a humidified atmosphere with 5.5% CO_2 at 37˚C. Cell dissociation was achieved with 0.1% trypsin-0.04% EDTA for the carcinoma cells and 0.05% trypsin-0.02% EDTA for the fibroblasts.

Chemicals. Ellagic acid (Sigma Chemical Co., St. Louis, MO, USA) was solubilized in dimethyl sulfoxide (DMSO), sonicated for three minutes, and prepared fresh daily. The stock concentration of 1% ellagic acid was high enough so that when diluted prior to use, the residual concentration of DMSO was not cytotoxic. For use in the studies, ellagic acid was directly introduced into exposure medium DMEM lacking pyruvate (cat. no. 11995-065), 10% Serum Plus (JRH Biosciences, Lenexa, KS, USA), 2% FBS, and antibiotics, followed by vortexing; fresh solutions were made for each experiment.

Cell proliferation assay. Individual wells of a 96-well microtiter tissue culture plate were inoculated with 0.2 ml of the growth medium containing 2×10^4 cells/well for HSC-2 cells and 1.5×10^4 cells/well for the HF-1 fibroblasts. After a day of incubation, the growth medium was removed and replaced with exposure medium, with or without ellagic acid at different concentrations. In some studies, the cells were coexposed to ellagic acid and 110 mg/l pyruvate. One complete row, *i.e.* eight wells, was used for each concentration of test agent. After exposure to the test agents for one to three days, viability was assessed with the neutral red (NR) assay, according to previously described procedures (3-5).

Hydrogen peroxide (H_2O_2) assay. Measurement of H_2O_2 was carried out by the ferrous ion oxidation xylenol orange (FOX) method using the PeroXOquant Quantitative Peroxide Assay Kit, lipid-soluble formulation (Pierce Biotechnology, Rockford, IL, USA) as previously described (14).

Intracellular glutathione assay. Cells, maintained in growth medium and grown to about 80% confluence in 60 mm tissue culture plates, were washed with phosphate buffered saline (PBS) and treated for 4 h in serum-free DMEM containing 50, 100, or 150 μM ellagic acid. Three plates of cells were used per concentration of ellagic acid. Measurements of intracellular reduced glutathione (GSH) were performed according to the procedures of Weisburg *et al.* (3).

Apoptosis assay: Flow cytometry. HSC-2 cells, grown to ~80% confluence in 60 mm plates, were untreated or treated for 72 h with different concentrations of ellagic acid. Afterwards, the cells were washed with PBS, typsinized, and diluted to a density just below 5×10^5 cells/ml. A 20 μl sample of cells, added to 380 μl of Guava ViaCount Reagent (Millipore, Billierica, MA, USA), was placed on ice in the dark for 5 min. Cell viability, apoptosis, and cell death were determined with a Guava Easycyte Miniflow Cytometer (Millipore). The Guava Viacount Reagent distinguishes between viable, apoptotic, and nonviable cells based on the differential permeability of its DNA binding dyes. The fluorescence of each dye is resolved operationally to allow for the quantitative assessment of viable, apoptotic, and non-viable cells present in a suspension (15).

Apoptosis assay: Western blotting. HSC-2 cells grown to ~80% confluence were untreated or treated for 48 h with ellagic acid in exposure medium. Cells were lysed with RIPA buffer (Thermo Scientific, Rockford, IL, USA), containing complete EDTA-free protease inhibitor (Roche Diagnostics Corp., Indianapolis, IN, USA), and then centrifuged (12,000×*g*, 10 min) to remove cellular debris. Protein concentrations of the cell lysates were quantified with the BCA Protein Assay Kit (Pierce Biotechnology). Equal concentrations of total protein from each sample were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [10% for poly ADP ribose polymerase (PARP) detection; 4-20% for caspase-3 detection] (Thermo Scientific), electroblotted to nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA), and blocked with 5% dry milk in Tris-buffered saline containing 0.1% Tween 20. Membranes were then probed with polyclonal antibody to PARP (rabbit antiserum, diluted 1:5,000; Abcam, Cambridge, MA, USA) to detect both full-length and cleaved PARP, or with anti-caspase-3 (rabbit antiserum, Cell Signaling Technology, Danvers, MA, USA). The portion of the membrane corresponding to the molecular weight of actin was probed with a rabbit antibody to actin (rabbit antiserum, diluted 1:7,500; Santa-Cruz Biotechnology, Inc., Santa Cruz, CA, USA) to ensure equal protein loading. Following incubation with the appropriate peroxidase-conjugated secondary antibodies (1:5,000 dilution), membranes were developed using the ECL detection kit (Amersham). Western blot analyses were performed a minimum of three times (4).

Apoptosis assay: Caspase 3 and 7 activation (luminescence). HSC-2 cells, seeded into a 96-well white-walled microtiter tissue culture plate, were treated for 48 h with ellagic acid. Cells were lysed and caspase activity was determined by adding the Caspase-Glo 3/7 reagent (Promega, Madison, WI, USA) containing the proluminescent caspase-3/7 substrate. Cleavage of the substrate by active caspases present in the cell lysate resulted in a luminescent signal, measured with a Promega Glomax Multi Detection luminometer (Promega, Madison, WI, USA).

Microscopy. A: Brightfield microscopy for antioxidant property of ellagic acid. Cells were seeded onto coverslips in 35 mm diameter tissue culture dishes containing growth medium. After the

monolayers reached 70% confluence, the cells were untreated for 6 h (control) in growth medium or treated for 6 h in medium amended with 33.1 μM ellagic acid, 175 μM gallic acid [a pro-oxidant polyphenol (15)], or a combination of ellagic and gallic acids. Thereafter, the cells were washed in PBS, fixed in methanol, stained with aceto-orcein, and viewed with a brightfield microscope observing changes in cell density and morphology.

B: Fluorescent detection of apoptotic cell morphology. As described above, cells on coverslips were untreated or treated with 175 μM ellagic acid for 48 h, stained with acridine orange (4 μg/ml in PBS), and viewed under a fluorescent microscope observing morphological abnormalities, such as cell blebbing and hypercondensation of nuclei.

C: Fluorescent detection of intracellular reactive oxygen species (ROS). Cells, grown on coverslips until approximately 80% confluence, were washed with PBS and treated with exposure media (serum-free DMEM, without pyruvate and phenol red) amended with different concentrations of ellagic acid, H_2O_2, or a combination of ellagic acid and H_2O_2, for 4 h at 37˚C. A 10 mM stock solution of 2',7'-dichlorodihydrofluorescein diacetate (DCHF-DA) was prepared by dissolving the powder in DMSO and then diluting it with warm exposure medium to 20 μM. HSC-2 cells, washed with PBS, were treated with DCHF-DA for 30 min at 37˚C. Thereafter, the extracellular DCHF-DA was removed and the cells were incubated for an additional 30 min at 37˚C in the presence of warm exposure medium. During this time, the internalized dye was hydrolyzed by intracellular esterases and the resultant dichlorodihydrofluorescein was oxidized by intracellular ROS to produce a fluorescent product, dichlorofluorescein (DCF). Following this incubation, the cells were washed with PBS, fixed with 3.7% formaldehyde in PBS, air dried, and attached to slides. The slides were observed with a Zeiss microscope equipped with a filter set capable of detecting DCF with an absorption maximum of 492 nm and a fluorescent emission maximum of 517 nm. The intensity of fluorescence, indicative of intracellular ROS, was compared in untreated control cells and in cells treated with ellagic acid alone, H_2O_2 alone, and with a combination of ellagic acid and H_2O_2.

Statistics. All experiments were performed a minimum of three times. Data for the FOX assay are presented as the arithmetic mean±standard errors of the mean (S.E.M.) and those for the cytotoxicity assay are presented as the mean arithmetic percentages relative to the control±S.E.M. Experimental data were analyzed with a one-way analysis of variance (ANOVA) followed by Tukey's multiple range test for significant differences. The *p*-value of the effect had to be ≤0.05 to be considered significant.

Results

Cytotoxicity assays. Preliminary studies compared the responses of carcinoma HSC-2 cells and normal gingival HF-1 fibroblasts to ellagic acid. Ellagic acid, at concentrations up to 200 μM, was nontoxic to HSC-2 and HF-1 cells after a one-day exposure. At longer exposure times, differential sensitivities to the two cell types were noted. Toxicity towards the HSC-2 cells, but not towards the fibroblasts, was noted after two- and three-day exposures to ellagic acid. For the HSC-2 cells, the midpoint cytotoxicity (NR_{50}) values for two- and three-day exposures to ellagic acid were estimated at 260 and 142 μM, respectively (Figure 2A). The HF-1 cells were resistant to 200 μM ellagic acid for similar exposure times (Figure 2B). As the fibroblasts were unaffected by ellagic acid, at the concentrations and exposure times studied, further studies were directed to HSC-2 cells only.

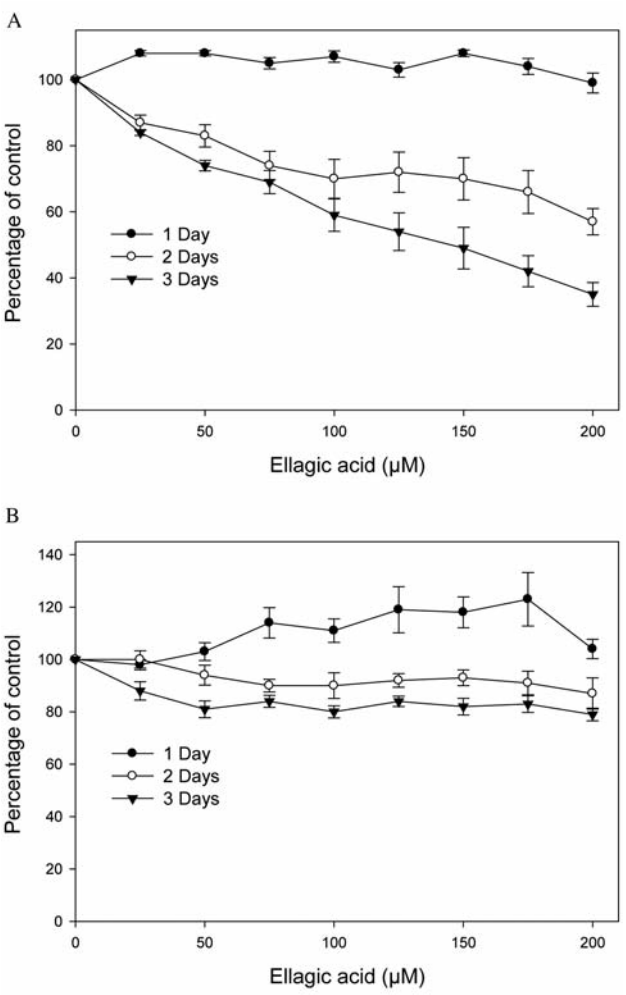

Figure 2. *Comparative responses of human carcinoma HSC-2 cells (A) and normal human gingival HF-1 fibroblasts (B) to ellagic acid, after one, two and three days of exposure. Cytotoxicity was assessed with the neutral red assay. The data are presented as the arithmetic mean percentage of control±standard error of the mean.*

Pro-oxidant studies. The generation of H_2O_2 in cell culture exposure medium amended with 50 to 200 μM ellagic acid for 4 h was determined with the FOX assay. Although a progressive increase in H_2O_2 was detected as the concentration of ellagic acid was increased, the overall generation of H_2O_2 was relatively minor. For comparative purposes, H_2O_2 was quantified in medium amended with similar concentrations of

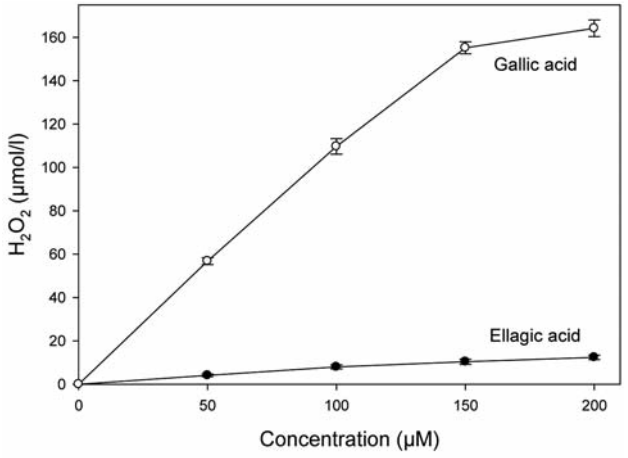

Figure 3. *Generation of hydrogen peroxide, as determined by the ferrous ion oxidation xylenol orange (FOX) assay, in cell culture exposure medium amended with different concentrations of ellagic acid and of gallic acid. The data are expressed as the arithmetic mean±S.E.M.*

gallic acid, another nutraceutical polyphenol but with a strong pro-oxidant property (16) (Figure 3).

To confirm that the level of H_2O_2 generated by ellagic acid was not significant enough to promote oxidative stress, the intracellular level of GSH was monitored in HSC-2 cells treated for 4 h with ellagic acid. The background level in nontreated HSC-2 cells was 33 nmoles of GSH/10^6 cells and in cells exposed to 50, 100, and 150 μM ellagic acid it was 35, 32, and 32 nmoles of GSH/10^6 cells, respectively. Pyruvate, a scavenger of H_2O_2 which protects cells from pro-oxidant polyphenols (14), had no protective effect at a concentration of 110 mg/l on ellagic acid up to 200 μM, as determined with the NR assay (data not shown).

Antioxidant studies. To ascertain the antioxidant property of ellagic acid, DCHF-DA was used to monitor intracellular ROS in HSC-2 cells exposed for 4 h to 33.1 μM ellagic acid alone, 200 μM H_2O_2 alone, and a combination of H_2O_2 and ellagic acid. Minimal fluorescence, indicating the background level of intracellular ROS, was detected in untreated control cells and in cells exposed to 33.1 μM ellagic acid alone. HSC-2 cells treated for 4 h with H_2O_2 fluoresced brightly, whereas only background fluorescence was noted in cells coexposed to ellagic acid and H_2O_2 (Figure 4A and B).

To further evaluate the antioxidant nature of ellagic acid, HSC-2 cells were exposed for 6 h to 33.1 μM ellagic acid alone, to a cytotoxic concentration of gallic acid alone, *i.e.* 125 μM, and to a combination of ellagic acid and gallic acid. The cytotoxicity of gallic acid was correlated to its pro-oxidant nature (17). No differences in cell density and morphology were noted in cells exposed to ellagic acid as compared to untreated control cells. A lesser cell density and aberrant cell morphologies were noted upon treatment with gallic acid. However, as compared to the control, cell density and cell morphology were unaffected in samples co-treated with gallic and ellagic acids (Figure 4C-F).

Apoptosis studies. The proapoptotic effect of ellagic acid on HSC-2 cells was evaluated using several approaches. Flow cytometric analyses of HSC-2 cells exposed for two days to ellagic acid showed that increasing the concentration of ellagic acid from 50 to 150 μM progressively increased the numbers of apoptotic cells (Figure 5A and B). Cells treated with 175 μM ellagic acid for 48 h and stained with acridine orange exhibited morphological changes characteristic of apoptosis, including cell shrinkage, dense cytoplasm and chromatin, and blebbing at the cell surface (Figure 6). Immunoblot analyses of lysates from HSC-2 cells treated with 100 to 175 μM ellagic acid for 48 h showed activation of caspase-3 and cleavage of PARP. Caspase-3 is a key executioner of apoptosis and its activation was indicated by cleavage of the proenzyme at aspartic acid 175, yielding 17/19 kDa and 12 kDa active products. Another marker of irreversible apoptotic cell death is the cleavage and, thereby, inactivation of PARP by caspases (Figure 7A). Increasing the concentration of ellagic acid from 50 to 175 μM progressively increased the activities of caspase-3 and caspase-7, as detected with a luminescence assay. The slight decrease in caspase activities at 200 μM ellagic reflected the lower cell viability at this concentration (Figure 7B).

Discussion

Many studies with human cells in culture have evaluated the potential anticancer effects of dietary plant-derived polyphenols, showing that the polyphenols, acting as pro-oxidants, generate sufficient levels of ROS to induce oxidative stress, leading to apoptosis of carcinoma cells. Studies, albeit limited, that compared carcinoma to normal cells showed the normal cells to be less sensitive to pro-oxidant polyphenols than the cancer cells (3, 4, 15). Sakagami in reviewing research on the comparative responses of oral carcinoma cells and gingival fibroblasts to natural plant-derived products concluded that the apoptosis-inducing activity of these test agents, many of which were polyphenols, reflected an undefined tumor-specific activity against oral squamous carcinoma cells (1). As many of these polyphenols exerted pro-oxidant activity, at a level significant enough to induce oxidative stress, a suggested explanation was that the greater sensitivity of cancer cells than of normal cells to pro-oxidant polyphenols reflected their compromised cellular redox status and deficient antioxidant defenses (3, 4, 15). As noted in the FOX assay, ellagic acid was a weak generator of H_2O_2. In the HSC-2 cell-based assays, the level of pro-oxidants produced by ellagic acid was inefficient to reduce the intracellular level of GSH, the main cellular defense against oxidative stress, and co-treatment with pyruvate, a

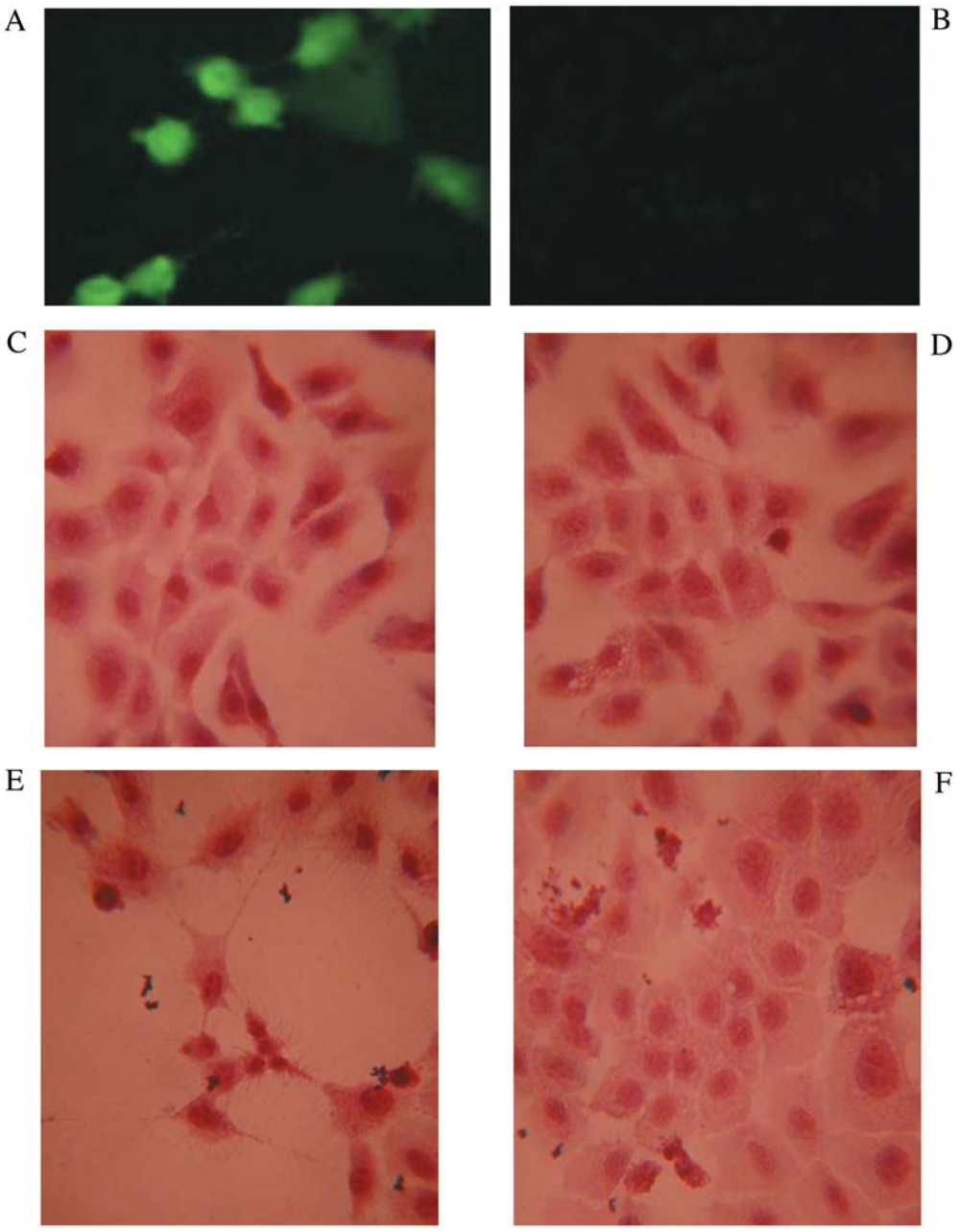

Figure 4. *Antioxidant property of ellagic acid. HSC-2 cells were stained with 20 μM 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) for 30 min following a 4 h exposure to 200 μM H_2O_2 (A) or 200 μM H_2O_2 in the presence of 33.1 μM ellagic acid (B). Fluorescence is indicative of intracellular ROS. The absence of intracellular fluorescence was noted in untreated (control) HSC-2 cells and in HSC-2 cells treated with 33.1 μM ellagic acid alone. HSC-2 cells were untreated (C), and treated for 6 h with 33.1 μM ellagic acid (D), 125 μM gallic acid (E), or a combination of ellagic acid and gallic acid (F). Cells were stained with aceto-orcein and viewed with a brightfield microscope. Magnification, ×320.*

scavenger of H_2O_2, had no effect on the cytotoxicity of ellagic acid. Apparently, the differential sensitivity of HSC-2 and HF-1 cells to ellagic acid was not a function of their differential intracellular defensives towards oxidative stress. However, Han *et al*. showed Chinese hamster lung V79-4 cells treated with ellagic acid exhibited elevated activities of superoxide dismutase, catalase, and glutathione peroxidase (12). Yet no evidence of ellagic acid acting as a pro-oxidant, *i.e*. as the stimulant to activate these antioxidant activities, was presented. Perhaps the low level of H_2O_2 generated by ellagic acid as shown in the studies herein with the FOX assay was sufficient to activate the antioxidant enzymatic activities in the V79-4

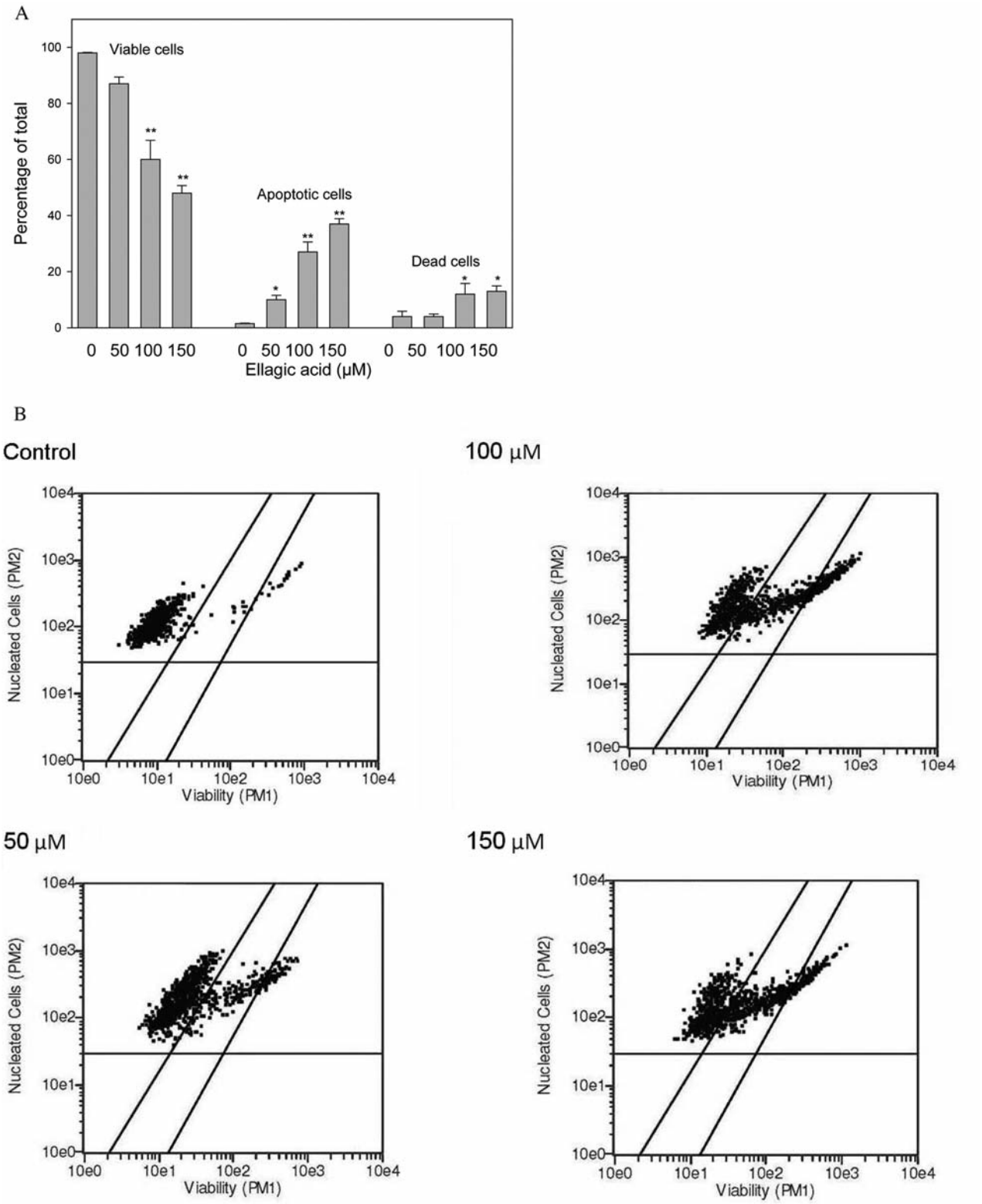

Figure 5. *A: Cytometric analysis, using Guava ViaCount Reagent, of a 72 h exposure of HSC-2 cells to increasing concentrations of ellagic acid. The percentage of viable, apoptotic, and dead cells were quantified by the flow cytometer. The data are expressed as the arithmetic mean percentage±S.E.M. of viable, apoptotic, and dead cells; *p≤0.05 and **p≤0.01 as compared to untreated cells. B: Representative FACS profiles of the viability and apoptosis of HSC-2 cells after a 72 h treatment with different concentrations of ellagic acid. Viable cells are located on the left side of each panel; apoptotic cells between the two lines; and dead cells on the right side of the panel.*

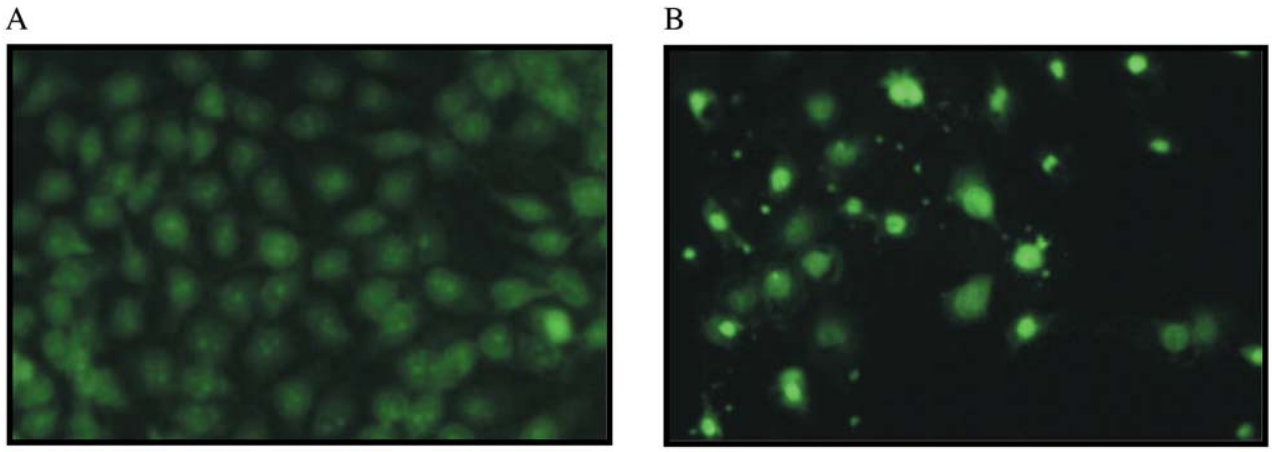

Figure 6. *Fluorescence microscopy of HSC-2 cells, untreated (A) and treated for 48 h (B) with 175 μM ellagic acid and stained with acridine orange. Magnification, ×320.*

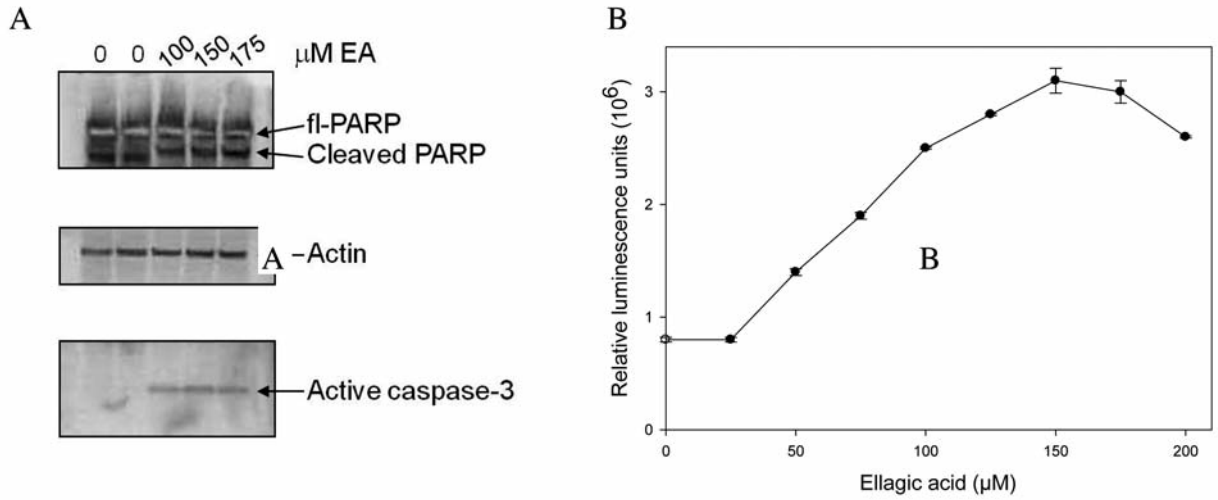

Figure 7. *A: Western blot showing full length (fl) and cleaved poly ADP ribose polymerase (PARP) and activation of caspase 3, both markers of apoptosis, in lysates of HSC-2 cells treated for 48 h with different concentrations of ellagic acid (EA). B: Induction of caspase 3 and 7 in HSC-2 cells treated for 48 h with different concentrations of EA. Activity was measured luminometrically. The data are expressed as the arithmetic mean luminescence units±S.E.M; all data points at 50 μM ellagic acid and above derived p-values ≤0.01 as compared to untreated cells.*

cells. Another approach to explain the differential sensitivities was provided by Losso *et al*. (8), who noted that several human cancer cell lines, including Caco-2 (colon), MCF-7 and Hs 578T (both breast), and DU 145 (prostate gland), were more sensitive to ellagic acid than were normal human HEL-299 lung fibroblasts. Their research suggested that this differential sensitivity was attributed to a reduction of ATP specifically in ellagic acid-stressed cancer cells, but not in normal fibroblasts.

Intracellular ROS in HSC-2 cells treated with H_2O_2 were eliminated in the presence of ellagic acid. Similarly, Festa *et al*. (6) observed similar findings with Chinese hamster ovary cells co-treated with H_2O_2 and ellagic acid. Furthermore, using the alkaline Comet assay, Festa *et al*. (6) noted that DNA damage in Chinese hamster ovary cells treated with either H_2O_2 or bleomycin, a generator of ROS, was markedly reduced upon cotreatment with ellagic acid. Han *et al*. observed that ellagic acid inhibited lipid peroxidation in Chinese hamster lung V79-4 fibroblasts treated with H_2O_2 and, in a cell-free assay, showed that ellagic acid exhibited 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (12). The cytotoxicity of gallic acid, a strong pro-oxidant (16), was correlated to its pro-oxidant nature (17). The cytotoxicity of gallic acid towards HSC-2 cells was lessened in the presence of a non-cytotoxic concentration of ellagic acid. Pavlica and

Gebhardt similarly noted that the cytotoxicity of H_2O_2 and of t-butyl hydroperoxide to neuronal PC12 cells was suppressed by ellagic acid (18).

Induction of apoptosis in HSC-2 cells exposed to ellagic acid was shown by various apoptosis-indicators, including the appearance of apoptotic cell morphology, flow cytometry to differentiate viable, apoptotic, and dead cells, activation of caspase-3/7 shown by luminescence; and immunoblot detection of the conversion of procaspase-3 to activated caspase-3 and of the cleavage of PARP, a substrate of caspase-3. The proapoptotic characteristic of ellagic acid has been noted by others and have included other indicators of apoptosis, such as internucleosomal chromosomal DNA degradation (7, 10, 12, 13), stimulation of the mitochondrial apoptotic pathway associated with mitochondrial depolarization, cytochrome *c* release, and downstream caspase activation (7); effects on signaling pathways, including increased levels of Bcl-2–associated X protein, a proapoptotic protein (12) and inhibition of nuclear factor κB, an anti-apoptotic protein (7); and by blockage of apoptosis by the pan-caspase inhibitor, Z-VAD-FMK (9). Other molecular aspects of ellagic acid cytotoxicity include cell growth arrest at the S and G_2/M phases of the cell cycle (11), reduced levels of pro-matrix metalloproteinases (8), and alteration of the expression levels of mitogen-activated protein kinases signaling genes, specifically leading to deactivating the extracellular signal-regulated kinase signaling pathway which influences cell proliferation and growth (11).

In summary, this research demonstrated that HSC-2 carcinoma cells were more sensitive to ellagic acid than were normal gingival fibroblasts. Regarding the HSC-2 cells, it was also shown that the pro-oxidant nature of ellagic acid was not significant enough to induce oxidative stress, that the antioxidative property of ellagic acid sequestered intracellular ROS in cells challenged with H_2O_2 and protected against challenge with gallic acid, a pro-oxidant polyphenol, and that ellagic acid induced apoptosis.

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