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Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 cells

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We demonstrate that two hydroxycinnamic acids, (*E*)-ferulic acid and (*E*)-*p*-coumaric acid, have the ability to protect against oxidative stress and genotoxicity in cultured mammalian cells. They also show the ability to reduce the activity of the xenobiotic metabolising enzyme, cytochrome P450 1A, and downregulate the expression of the cyclooxygenase-2 enzyme. At equitoxic doses, their activities are equal to or superior to that of the known anticarcinogen, curcumin. The hydroxycinnamic acids are both important components of plant cell walls in certain plant foods. It is known that the action of microbial hydroxycinnamoyl esterases can lead to the release of hydroxycinnamic acids from ester-linkages to cell wall polysaccharides into the human colon. Thus, providing they can reach effective levels in the colon, they could provide an important mechanism by which dietary fibres of food plants, such as spinach or cereal, protect against colon cancer.

Keywords: Antimutagen / Antioxidant / Curcumin / Dietary fibre / Ferulic acid / Hydroxycinnamic acid / *p*-Coumaric acid

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1 Introduction

Although the original concept of dietary fibre (DF) regarded this as plant cell walls, more recent definitions have focused on the chemistry and physiological properties [1, 2]. In particular, the polysaccharide composition has been considered important, so that in the UK, for example, DF has been defined as non-starch polysaccharides [3]. However, plant cell walls contain other components that may be relevant to health protection. In addition to polysaccharides, the cell walls of cereal grains and a number of other food plants (*e.g.*, bananas, beetroot, pineapple, spinach) contain ferulic acid (~0.5–2% dry matter), mostly as the *trans*-isomer, ester-linked to specific polysaccharides [4–10]. These cell walls also contain small amounts of *p*-coumaric acid, again mostly as the *trans*-isomer. In cereal cell walls, the ferulic acid is ester-linked to arabinoxylan polysaccharides, but in beetroot and spinach it is linked to pectic polysaccharides. Recently, a series of dimers of ferulic acid have been identified in cell walls containing ferulic and *p*-coumaric acids [11, 12]. These dimers are formed by oxidative dimerization and are referred to as dehydrodimers of ferulic acid.

Both ferulic acid and its dehydrodimers have shown *in vitro* free radical scavenging properties [13–16]. Also, we have shown that ferulic acid, *p*-coumaric acid and 5,5′-dehydrodiferulic acid are antimutagens in bacterial assays [17]. If such results extrapolate to mammalian cells, this suggests that these compounds could act as antimutagens in protecting against degenerative diseases such as cancer. It is of interest to ask whether they protect against DNA breakage and/or chromosome breaks. Other activities of phenolic compounds might also have relevance for protection against cancer. In a previous study of rats treated with the carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), inclusion of 10% wheat bran in the diet led to an apparent reduction in IQ metabolites but not of intact IQ in plasma [18]. Wheat bran supplementation showed differential effects on phase I enzymes, significantly increasing the activity of hepatic cytochrome P450 (CYP) isozyme CYP3A2 ($P < 0.005$), but slightly reducing the activity of CYP1A1/2. We suggested that modulation of activity of xenobiotic metabolising enzymes (XMEs) may be an important component of cancer protec-

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Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; **COMET**, single cell gel electrophoresis assay; **COX-2**, cyclooxygenase-2; **CYP**, cytochrome P450; **DF**, dietary fibre; **GST**, glutathione *S*-transferase; **IQ**, 2-amino-3-methylimidazo[4,5-*f*]quinoline; **MN**, cytokinesis-blocked micronucleus; **TRAP**, total reactive antioxidant potential; **Trolox**, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; **XME**, xenobiotic metabolising enzyme

tion by wheat bran, and this effect may relate to the phenolic components rather than non-starch polysaccharides.

Cyclooxygenase-2 (COX-2) is an inducible isoform of prostaglandin H synthase which mediates prostaglandin synthesis during inflammation, and is selectively overexpressed in colon tumours [19]. Inhibition of COX-2 appears to reduce the incidence of colon cancer [20]. It has been suggested that reactive oxygen intermediates play a role in the regulation and expression of COX-2 [21]. These authors demonstrated that a range of antioxidants both reduced the expression of COX-2 and modulated the growth of transplantable tumours in mice. Although the phenolic compounds occurring in DFs have not been studied for effects on COX-2, there are data available on the related compound, curcumin. This is a phenolic compound that shows structural analogies to the dehydrodimers of ferulic acid in certain DF. It acts as a potent anti-inflammatory agent through inhibition of prostaglandin synthesis [22], and as a weak direct inhibitor of the enzyme activity of COX-2 [23].

In the present study, we tested the hypothesis that the hydroxycinnamic acids that occur in plant cell walls can act as antioxidants, protect against DNA breakage or clastogenesis in mammalian cells, and/or modulate the activity of certain enzymes that are relevant to carcinogenesis. The chemicals tested were (*E*)-ferulic acid (Fig. 1 a) and (*E*)-*p*-coumaric acid (Fig. 1 b). The activities of the hydroxycinnamic acids were compared with those of the known anticarcinogen, curcumin (Fig. 1 c). An *in vitro* assay for total reactive antioxidant potential (TRAP) was used to compare chain-breaking antioxidant potential [24] of the compounds. DNA strand breaks at the level of single cells were measured using the single cell gel electrophoresis (COMET) assay [25] and clastogenesis estimated using the cytokinesis-blocked micronucleus (MN) assay [26]. Two known mutagens, hydrogen peroxide and IQ, were used to induce DNA breakage or clastogenesis in these cell culture experiments. We also tested the ability of all compounds to modulate the activity of two XMEs, glutathione *S*-transferase (GST) and CYP1A1/2, and also the expression of COX-2 enzymes, after long-term exposure in mammalian cells.

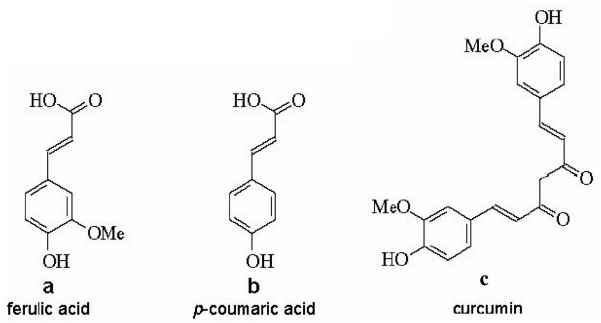

Figure 1. Structures of hydroxycinnamic acids and of curcumin. (a) Ferulic acid. (b) *p*-Coumaric acid. (c) Curcumin.

2 Materials and methods

2.1 Chemicals

(*E*)-Ferulic and *p*-coumaric acids were obtained as 99% pure from Sigma Chemical (St. Louis, MO, USA), while curcumin and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were >90% pure, from Aldrich Chemical Company (Milwaukee, WI, USA). IQ (99% pure) was from Toronto Research Chemicals (Downsview, Ontario, Canada). Glutathione was obtained from Sigma, St. Louis, MO, USA. Ethoxyresorufin, resorufin and 1-Chloro-2,4-dinitrobenzene (CDNB) were also purchased from Sigma. NADPH was from Boehringer Mannheim (Auckland, NZ). Anti-CYP1A1/2 was purchased from Gentest Corporation (Waburn, MA, USA).

2.2 Cell lines and tissue culture

All the cell lines (HT-29, EMT6, SW620, LOVO, and HCT-8) were the kind gift of Dr. G. Finlay, Auckland Cancer Society Research Centre, The University of Auckland. They were selected to represent a range of p53 genotypes and metabolic status. Cells were routinely maintained in α-minimal essential medium with 10% foetal calf serum (α-MEM) under standard conditions of 5% CO_2 in air at 37°C in a humidified incubator. For micronucleus studies, exponential phase cultures were initiated 24 h previous to experiments at 1.5×10^6 cells/dish in 100 mL dishes containing 10 mL α-MEM. Increasing concentrations of the chemicals were added in 5 mL prewarmed medium, and cells were incubated at 37°C for 60 min. Treatment was terminated by washing cells twice with phosphate-buffered saline (PBS). Immediately following treatment, adherent cells were trypsinised, diluted, and plated for estimation of cytotoxicity, or fresh growth medium was added and cells grown for further time intervals before micronucleus assays. For XME assays and the COMET assay, HT-29 cells were subcultured into P60 plates at a concentration of 700 cells/mL. After 3 h settlement, a designated concentration of compound, dissolved in <1% DMSO, was added to the plate. The media was changed on the fourth day with the same concentration of the same compound in it, then the cells harvested on the seventh day.

2.3 Growth inhibition assay

The effects of the test compounds on the growth of human cancer cell lines were evaluated using a colorimetric assay [27]. Cells were routinely maintained as above. Cells were subcultured weekly and, according to their growth profiles, the optimal plating densities of each cell line were determined to ensure exponential growth throughout the experimental period. Cells were inoculated into 96-well microtiter

plates in 150 μL at plating densities depending on the doubling time of individual cell lines. After cell inoculation, the plates were incubated for 3 h prior to addition of experimental compounds. Stock solutions of all drugs were in DMSO and diluted to the desired concentration in media prior to the assay. Additional six$^1/_2$ log serial dilutions were made to provide a total of seven drug concentrations plus control. The final concentration of DMSO (0.13%) showed no interference with the growth of the cell lines. Aliquots of 150 μL of the test drugs were added to the appropriate microtiter wells already containing 150 μL medium, resulting in the desired final concentrations. Microtiter plates were incubated for 72 h after which the assay was terminated by the addition of cold TCA. Plates were incubated for 60 min at 4°C and then washed three changes of a large value of tap water and air-dried. SRB (from Sigma Chemical) solution (100 μL) at 0.4% w/v in 1% acetic acid was added to each well, and plates were incubated for 30 min at room temperature. After staining, unbound dye was removed by washing four times with 1% acetic acid. Bound stain was then solubilized with 10 mM unbuffered Tris (200 μL/well), the plates were shaken for 15 min on a gyratory shaker, and absorbance was read at 515 nm on an automated microplate reader (Model ELx808; Bio-Tek Instruments, Winooski, VT, USA).

2.4 Free radical scavenging

The TRAP assay [24] was used. 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH, from Wako Pure Chemical Industries, Osaka, Japan) was used as the peroxy-radical generator to start the reaction. The final reaction mixture for the assay contained 75 μM 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate (ABTS, from Sigma Chemical) and 2 mM AAPH in 50 mM acetate buffer (pH 4.3). The reaction mixture was incubated at 45°C for 60 min and then brought to room temperature. 0.5 mL of the phenolic acid solutions dissolved in 50 mM acetate buffer (pH 4.3) was added to 2.5 mL of the reaction mixture in each cuvette. For the blank, 0.5 mL 50 mM acetate buffer (pH 4.3) was used in place of the sample. The decrease in absorbance at 734 nm after 15 min at 25°C elicited by the additive was measured (Hewlett-Packard, Palo Alto, CA, USA; diode array spectrometer 8452A). The ascorbate standard (ascorbic acid sodium salt, from Sigma Chemical) solutions were similarly assayed in same time. Final results were calculated using the differences between the initial reading and final reading compared to a calibration curve for known amounts of ascorbate.

2.5 COMET assay for DNA damage

The COMET assay was used to assess the stability of DNA. The assays and its variants are described more fully elsewhere [25]. Briefly, cells were harvested by standard procedures, then suspended in agarose on an agarose precoated microscope slide, and lysed in a solution containing 100 mM EDTA, 10 mM Tris and 2.5 M NaCl for 24 h at 4°C. The resulting nucleoids were denatured and electrophoresed for 17 min, each under alkaline conditions. The electrophoresis conditions were 25 V and 300 mA. The presence of breaks in the DNA allows it to extend towards the anode, resulting in a comet-like image when stained with ethidium bromide and visualized under a fluorescence microscope. This assay provided an overall measure of DNA breakage after various types of DNA damage. For all of these assays, tail moment is calculated as tail length × tail % DNA/100, and an increase in tail moment provides evidence of an increased number of strand breaks in the nucleoid DNA. All steps for the COMET slide preparation were carried out in diffused light to prevent additional DNA damage.

2.6 Micronuclei

A density of 1.5×10^6 cells was seeded into P100 plates, and incubated for 3 h to ensure the cells had attached to the surface. Then the plate was exposed to the compound in the designated concentration for 1 h in the incubator. Cells were then rinsed twice with PBS, followed by the addition of α/10/PS medium with 1 mM IQ in it. After incubating for another hour, the plate was rinsed with 5 × PBS twice, followed by the addition α/10/PS medium. The plates were incubated for 22 h. 18 h before harvest, cytochalasin B (Sigma Chemical Co.) was added to the cultures to give a final concentration of 4.5 μg per mL. Cultures were harvested using standard procedures involving centrifugation, swelling in hypotonic KCl and fixation in methanol/acetic acid prior to dropping on a slide. After the slides were air dried, cells were then stained with a 10% Diff-Quik Stain Set (Dade Behring, Newark, DE, USA). Binucleate cells were scored for MN, identified using the criteria of Fenech and co-workers [26]. At least two sets of 1000 cells have been scored, one from each harvest.

2.7 Enzyme assays

The cell pellets were lysed in 0.5 mL HEDG buffer (25 mM HEPS, 1.5 mM EDTA, 1 mM DTT, and 10% glycerol, pH 7.4) and incubated for 30 min on ice. Cytosol was prepared by differential centrifugation as the $106\,000 \times g$ supernatant. The pellet was resuspended in TE-G buffer (10 mM Tris, 1 mM EDTA, and 20% glycerol, pH 7.4) and denoted the microsome. Proteins were quantified by the Bradford method with Bradford reagent. Enzyme assays were done according to the protocols described by Helsby and co-workers [28].

2.7.1 Cytochrome P450 1A1/2

The *in vitro* metabolism of ethoxyresorufin by HT-29 cell microsome was used as a probe to determine CYP1A activity. Ethoxyresorufin (5 μM), NADPH (1 mM), and HT-29 cell microsomes (0.1 mg) were incubated in Tris buffer (0.05 M), pH 7.4 (1 mL final volume). Incubations were at 37°C for 10 min. The reaction was terminated with ice-cold methanol (1 mL) followed by centrifugation ($2000 \times g$, 10 min), the fluorescence of resorufin determined (excitation: 530 nm and emission 585 nm) and compared to a calibration curve for known amounts of resorufin.

2.7.2 Glutathione *S*-transferase

The conjugation of glutathione to CDNB was measured as a nonspecific substrate for GST activity. To a quartz cuvette was added glutathione (1 mM), CDNB (1 mM) in a final volume of 3 mL 100 mM potassium phosphate buffer (pH 6.5). The cuvette was placed in a spectrophotometer (Hewlett-Packard; diode array spectrometer 8452A) and the reaction initiated by the addition of HT-29 cell cytosol (1 mg). The increase in absorbance at 340 nm over a 3 min period was measured at room temperature. An extinction coefficient of 9.6 cm · mM^{-1} was used to determine activity from the initial slope of the reaction.

2.8 Western immunoblots

Cells were pelleted and treated as for XME. HT-29 cell cytosolic and microsomal samples were diluted to 1 mg · mL^{-1} with Milli-Q water and then further diluted 1 : 4 with sample buffer. Samples were then boiled at 70°C for 20 min. SDS-PAGE was performed on 10% acrylamide gels using the BioRad Mini-Protean III apparatus (Hercules, CA, USA). 20 μL sample was added to each lane and run for 40 min at 200 V. Proteins were then transferred at 110 V for 60 min onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Auckland, NZ). The PVDF was blocked overnight in 5% non-fat milk protein containing 0.4% foetal calf serum (FCS). The membrane was then probed with monoclonal antibodies to either COX-1 or COX-2 which were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary probing was performed with an Elite ABC Vectastain kit (Vector Labs, Burlingame, CA, USA) and the proteins visualised by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech UK, Amersham, UK) using a film exposure time of 60 s (Kodak Scientific Imaging Film, Rochester, NY, USA). Quantitative analysis of the bands (area/density relationships) was achieved by the MD30 image analysis system (Leading Edge, Adelaide, Australia).

2.9 Statistical analyses

Initial statistical analysis to evaluate differences among the groups was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. For data found to be unsuitable by ANOVA treatment, statistical analysis was performed using Student's *t*-test. Data were considered significant if $p < 0.05$.

3 Results

3.1 Cell growth inhibition by hydroxycinnamic acids and curcumin in various mammalian cell lines

The potential for growth inhibition of each of these chemicals on different cell lines is compared in Table 1. Data are expressed as IC50, the concentration necessary to reduce survival to 50% of the value in untreated cells. We defined a maximum dose for future experiments as being that which resulted in 90% cell survival. While there were minor variations in these values between cell lines, the concentrations necessary for biological activity varied significantly between the hydroxycinnamic acids and curcumin, in that the former required approximately 100-fold higher concentration of chemical to reduce cell growth to 10%, (or 10-fold higher to reduce this to 50%) as compared with the latter. The HT-29 cell line was selected as an example of a human colorectal cancer cell, and concentrations of all chemicals selected to provide a maximum reduction of 10% cell growth in further experiments.

Table 1. Cell growth inhibition by hydroxycinnamic acids and curcumin in various mammalian cells

	Ferulic acid	*p*-Coumaric acid	Curcumin
HT-29	1.21 ± 0.07	1.19 ± 0.04	0.12 ± 0.01
EMT-6	0.87 ± 0.09	0.74 ± 0.01	0.08 ± 0.00
SW-620	0.98 ± 0.12	0.76 ± 0.02	0.08 ± 0.01
LOVO	0.69 ± 0.10	0.75 ± 0.07	0.05 ± 0.00
HCT-8	1.12 ± 0.03	0.79 ± 0.04	0.08 ± 0.01

Data are expressed as IC50, the concentration necessary to reduce survival to 50% of the value in untreated cells, plus or minus the standard error of the mean. The data are from triplicate experiments. Drug concentration is in mM.

3.2 Ability of ferulic acid, *p*-coumaric acid, and curcumin to scavenge free radicals

These experiments were done in the concentration range identified for mammalian cells. The data (Fig. 2) reveal that each of the three compounds has good activity in the TRAP

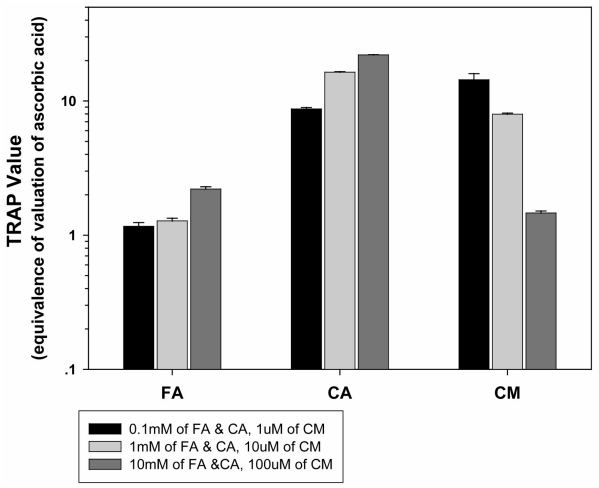

Figure 2. Effects of ferulic acid, *p*-coumaric acid, and curcumin on free radicals in the TRAP assay. Each data point represents mean ± SE from two experiments, each done in triplicate.

assay. While the activity of both hydroxycinnamic acids was increasing over the dose range tested, some pro-oxidant effects were starting to occur for curcumin. Data for the highest concentration of ferulic acid, all concentrations of *p*-coumaric acid and the two lower concentrations of curcumin all differed significantly ($p < 0.01$) from the Trolox standard.

3.3 Ability of hydroxycinnamic acids and curcumin to protect against DNA breakage in HT-29 cells

Figure 3 illustrates the effect of pregrowing cells with two different concentrations of ferulic and *p*-coumaric acids on DNA breakage induced by the presence of a set concentration (150 μM) of hydrogen peroxide. Significant differences ($p < 0.01$) from the control data are marked on the bars; there were also $p < 0.01$ levels of significance of the difference between the two concentrations tested of each chemical. Neither of the test chemicals induced DNA breakage when tested alone at these concentrations in this cell line (data not shown). Ferulic acid showed an ability to reduce DNA breakage by hydrogen peroxide, but only when a concentration of 1 mM was reached. However, this assay method fails to show protection by *p*-coumaric acid or curcumin, and may even imply that these compounds slightly enhance DNA breakage by hydrogen peroxide.

3.4 Ability of hydroxycinnamic acids and curcumin to protect against micronucleus formation in HT-29 cells

Figure 4 illustrates the dose response of ferulic and *p*-coumaric acids to show the effects of pregrowing cells for 7 days in the presence of the chemical, on micronucleus formation induced by 1 h treatment with a set concentration (1 mM) of the dietary carcinogen, IQ. Neither of the test chemicals induced micronuclei above spontaneous levels when tested alone at these concentrations in this cell line (data not shown). Both showed some ability to reduce micronucleus formation, but only when concentrations greater than 1 mM for ferulic and 3 mM for *p*-coumaric acid were reached; statistical significance of the change was only reached at the highest level tested of *p*-coumaric acid. Curcumin is also compared in these figures. These data provide no evidence for anticlastogenic effects of curcumin, at least in the concentration range tested.

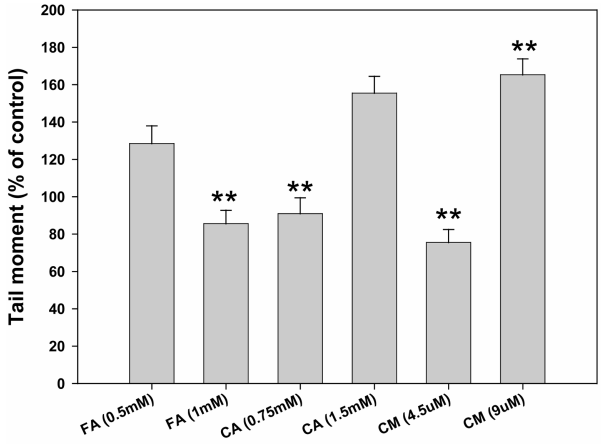

Figure 3. Ability of ferulic acid, *p*-coumaric acid and, curcumin to protect against DNA breakage induced by hydrogen peroxide in HT-29 cells, using the COMET assay. The data illustrates the mean tail moment of 100 cells at both concentrations of chemical tested. Cells were grown in the presence of each of these concentrations for 7 days, then washed, and treated with hydrogen peroxide for 10 min, before being harvested for COMET assay as described above. Each data point represents mean ± SE from two experiments, each done in triplicate.

3.5 Effects of hydroxycinnamic acids and curcumin on the activity of XMEs

The data illustrate that phase I enzymes are not coordinately regulated by hydroxycinnamic acids or curcumin in HT-29 cells. The activity of CYP1A, as measured by the *in vitro* metabolism of ethoxyresorufin, was decreased by each of the chemicals (Fig. 5). These changes in activity reached statistical significance ($p < 0.05$) for either concentration of ferulic acid or *p*-coumaric acid, but only the highest concentration of curcumin. It should, however, be noted that this concentration was approximately 100-fold lower than the concentration used for the other two chemicals. In contrast, the activity of the phase II enzyme GST, as measured by conjugation of CDNB, was not significantly changed (data not shown).

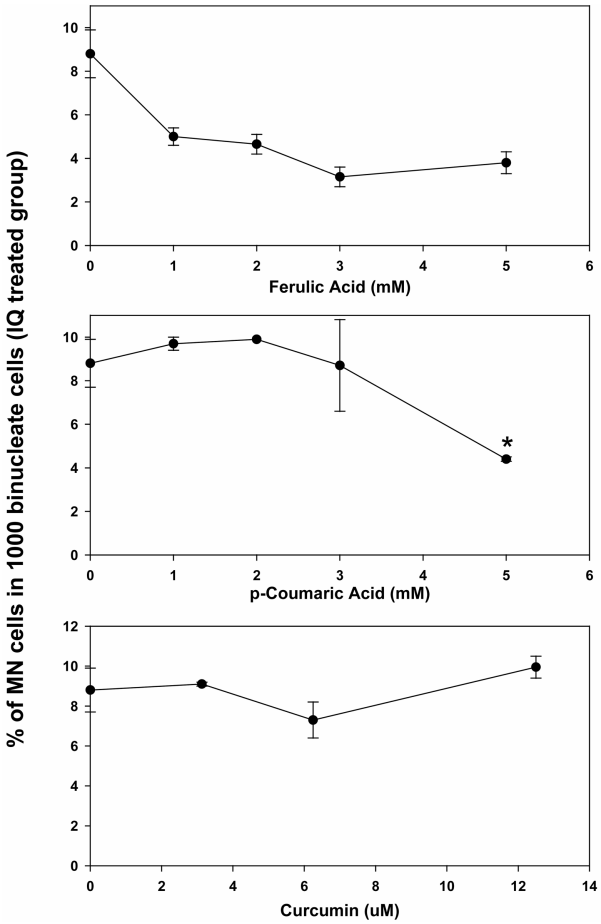

Figure 4. Ability of ferulic acid, *p*-coumaric acid, and curcumin to protect against micronucleus formation induced by the dietary carcinogen, IQ, in HT-29 cells. A concentration range of each phenolic was selected to span a range from nontoxic through to 90% survival, from preliminary experiments. Cells were grown in the presence of each of these concentrations for 7 days, then washed, and treated with IQ for 1 h. They were further grown as described in Section 2, before being harvested and stained for micronuclei. Each data point represents mean ± SE from two experiments, each done in triplicate.

3.6 Effects of hydroxycinnamic acids and curcumin on the expression of COX-2 enzymes in HT-29 cells

The expression of COX-2 enzymes in treated cells is expressed as a percentage of the expression in untreated cells in Fig. 6, while the Western blots are illustrated in Fig. 7. The level of expression of this enzyme is decreased by all treatments, but the changes in data reach statistical significance ($p < 0.01$) only for ferulic acid and curcumin at the highest concentrations tested.

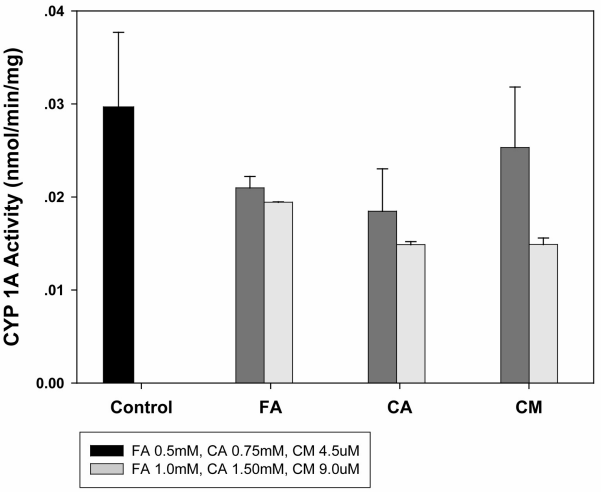

Figure 5. Effects of ferulic acid, *p*-coumaric acid, and curcumin on the activity of CYP1A in HT-29 cells. Two concentrations of each phenolic was tested, and compared with data for untreated cells. Data are normalised according to protein concentration. Control, black bar; low concentration, dark grey bars; high concentration, light grey bars. Each data point represents mean ± SE from two experiments, each done in triplicate.

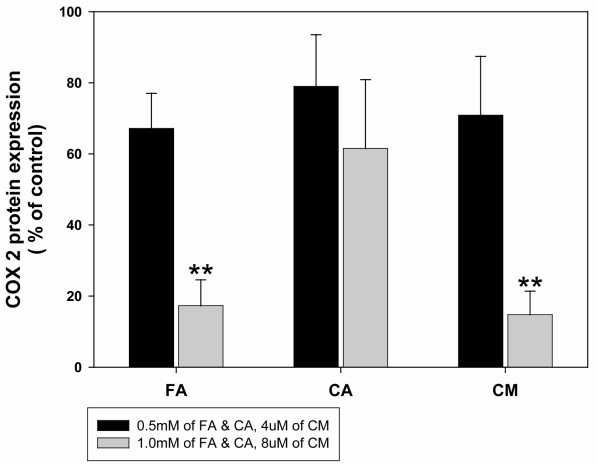

Figure 6. Effects of ferulic acid, *p*-coumaric acid, and curcumin on the expression of COX-2 enzymes in HT-29 cells. Two concentrations of each phenolic was tested, and compared with data for untreated cells. Data are normalised according to protein concentration. Low concentration, black bars; high concentration, light grey bars. Each data point represents mean ± SE from two experiments, each done in triplicate.

4 Discussion

This study has confirmed antioxidant effects of hydroxycinnamic acids [11, 13–16, 29, 30], and extended their range of known activities to include protection against DNA and chromosome breakage, as well as modulation of the activity of certain enzymes previously associated with

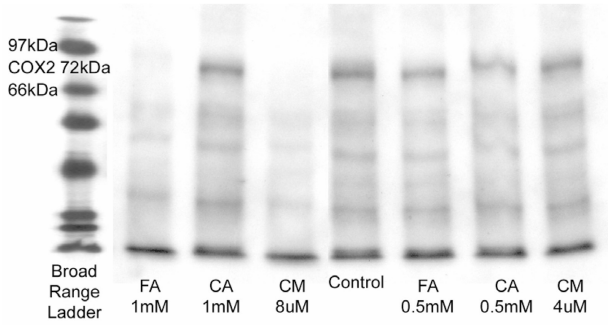

Figure 7. Western blot showing levels of COX-2 expression for cells in the presence and absence of phenolic compounds. Molecular weight markers are in the left hand lane, and the position of the COX-2 protein is indicated.

carcinogenesis. Given that the hydroxycinnamic acids are antioxidants, the simplest explanation of their inhibitory effect on hydrogen peroxide-peroxide induced DNA breakage is that they reduce the concentration of mutagenic oxidation products by free radical scavenging. However, free radical scavenging is unlikely to explain all the effects in the present study. Curcumin showed less evidence for anticlastogenic effects than did the hydroxycinnamic acids. Although curcumin shows protection against cancer in many studies, it has also been shown to be a co-carcinogen in other studies and at other tumour sites [31]. In the present studies, it was noteworthy that both *p*-coumaric acid and curcumin actually enhanced DNA breakage by hydrogen peroxide at high concentrations. It is possible that both compounds generate reactive oxygen species themselves as a result of pro-oxidant activity. We have previously commented that at different concentrations, curcumin may be able to act as either an antioxidant or as a pro-oxidant [17].

Modulation of the activity and expression of XMEs has been suggested to play a significant role in cancer protection [32]. At least hypothetically, an increase in phase II detoxification enzymes might be considered to beneficial, since this could enhance the excretion of carcinogens. Ferulic acid has been previously described as an *in vitro* inhibitor of ethoxyresorufin metabolism (CYP1A1/2) [33] and GST enzyme activity [34]. The present study confirmed the CYP1A1/2 activity of the compound, and suggested that either of the hydroxycinnamic acids, or the related compound, curcumin, could also have this effect. These studies also confirmed the activity of curcumin in reducing the expression of COX-2 enzymes, and extend the data to the hydroxycinnamic acids.

The hydroxycinnamic acids in cell walls are released when the cell walls are degraded by bacteria in the large intestine. For example, Buchanan and co-workers [35] demonstrated that when cell walls of spinach were fed to rats, the ester-linked feruloyl and *p*-coumaroyl groups were not released in the small intestine, but were released in the large intestine and absorbed from the gut. Furthermore, Kroon *et al*. [36] showed that incubation *in vitro* of sugar beet DF and wheat bran with human faecal bacteria resulted in the release of ferulic acid. The ferulic acid was rapidly and fully solubilized from the sugarbeet DF, but only partially and relatively slowly from the wheat bran. In wheat bran, most of the ferulic acid is ester-linked to arabinoxylans and occurs at high concentrations in aleurone cell walls [37, 38]**.** Although not lignified, these cell walls are only slowly and partially degraded by bacterial enzymes in rat and human large intestines [39 – 41]. In addition to the feruloyl and *p*-coumaroyl groups, the ferulic acid dehydrodimers are also released from cell walls following microbial degradation [42]. However, the concentrations of ferulic acid dehydrodimers in aleurone cell walls are low [38]. There is reason to believe that 95% of the total feruloyl groups are released into the colon in humans [36] thus making them bioavailable. They may be absorbed directly, or are susceptible to being converted to other compounds by colonic bacteria.

Cell wall release may lead to the compounds being more bioavailable in the colon than if the isolated compound is fed directly to animals or humans. Thus, for example, although rats fed ferulic acid displayed no changes in hepatic CYP levels [42], the regime used may not have resulted in such high levels in the colon as could be achieved through release of the acid from plant cell walls.

These studies extend the possible range of biological effects of plant cell wall phenolic compounds. These compounds occur in a range of food plants. In an accompanying paper in this volume, Bunzel and co-workers [43] describe how ferulic acid is attached to cell wall polysaccharides in the pseudocereal, amaranth. Since we know that >95% of cell-wall hydroxycinnamic acids are likely to be released in the human colon, it is possible that they could reach biologically significant levels in parts of the colon. The properties we have demonstrated support our previous suggestions [17, 43] that phenolic components of plant cell walls could have significance for dietary fibre protection against cancer. Thus, they would support the suggestion that an increased intake of food plants with cell walls rich in ferulic acid and its dehydrodimers may be beneficial in reducing the probability of cancer in human and animal populations.

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