Anti-cancer properties of phenolics from apple waste on colon carcinogenesis in vitro

M.J. McCann a,*, C.I.R. Gill a, G. O’Brien a, J.R. Rao b,c, W.C. McRoberts b, P. Hughes a, R. McEntee a, I.R. Rowland a

a Northern Ireland Centre for Food and Health (NICHE), Centre for Molecular Biosciences, University of Ulster, Coleraine, BT52 1SA Northern Ireland, United Kingdom
b Agri-Food Biosciences Institute (AFBI), Newforge Lane, Belfast, BT 9 5PX Northern Ireland, United Kingdom
c The Queen’s University of Belfast, United Kingdom

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Abstract

Colorectal cancer is one of the most common cancers in Western countries. The World Health Organisation identifies diet as a critical risk factor in the development and progression of this disease and the protective role of high levels of fruit and vegetable consumption. Several studies have shown that apples contain several phenolic compounds that are potent anti-oxidants in humans. However, little is known about other beneficial properties of apple phenolics in cancer. We have used the HT29, HT115 and CaCo-2 cell lines as in vitro models to examine the effect of apple phenolics (0.01–0.1% apple extract) on key stages of colorectal carcinogenesis, namely; DNA damage (Comet assay), colonic barrier function (TER assay), cell cycle progression (DNA content assay) and invasion (Matrigel assay). Our results indicate that a crude extract of apple phenolics can protect against DNA damage, improve barrier function and inhibit invasion (p < 0.05). The anti-invasive effects of the extract were enhanced with twenty-four hour pretreatment of cells (p < 0.05). We have shown that a crude apple extract from waste, rich in phenolic compounds, beneficially influences key stages of carcinogenesis in colon cells in vitro.

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1. Introduction

Colorectal cancer is one of the leading causes of cancer-associated deaths in the Western world. It is the third most common cancer in men (after prostate and lung) and the second most common in women (after breast). Diet and lifestyle are thought to be major risk factors for developing colorectal cancer (Parkin et al., 2005). It is thought that a healthy diet high in fruit and vegetables could significantly alter the incidence of 50–80% of colorectal cancers (Parkin et al., 2005; Potter, 1997). Recent reviews of the evidence for the protective effect of fruit and vegetables on cancer risk highlighted the uncertainty as to whether or not fruits are protective against colorectal cancer, as several case control studies have shown a protective effect, whilst cohort studies have been inconsistent (Riboli and Norat, 2003; Marques-Vidal et al., 2006; Williamson and Manach, 2005).

Polyphenolic compounds are an integral part of the human diet due to their wide abundance in fruits and vegetables and have attracted considerable interest as potential chemotherapeutic compounds (Boyer and Liu, 2004;
Kelloff et al., 2000; Terry et al., 2001; Lambert et al., 2005). Apples are one of the more common fruits consumed on a regular basis by many cultures (Boyer and Liu, 2004). It has been estimated that 22% of the dietary phenolics consumed in the USA are from apples (Vinson et al., 2001).

The protective effects of apples have been attributed primarily to their anti-oxidant properties. Apples have been shown to contain several phytochemicals that are thought to be protective in cancer: these include carotenoids, flavonoids, isoflavonoids, phenolic acids and lignans (Liu, 2004; van der Sluis et al., 1997; Ebeler et al., 2002; Weyant et al., 2001; Kasai et al., 2000; Lee et al., 2003; van der Woude et al., 2003; Sun et al., 2002; Wolfe et al., 2003; Eberhardt et al., 2000). Eberhardt et al. (2000) determined the phytochemical content of 100 g of apples and found that flavonoids and phenolics were more abundant in apples retaining their skins. In addition, the anti-oxidant capacity of apples (relative to Vitamin C) was higher in apples with skins than without (Eberhardt et al., 2000). It should be noted that while apples contain vitamin C (mean 12.8 mg/100 g), it only contributes 1% to total anti-oxidant capacity observed (Lee et al., 2003). Phytochemicals present in apples were found to contribute over 80% of the anti-oxidant capacity of the cultivars used (Lee et al., 2003). Sun et al. (2002) measured the phenolic content of a wide range of commonly consumed fruits and found that apples had the second highest phenolic content after cranberries. Apples also have a higher proportion of unbound or free phenols than other common fruits such as oranges and strawberries. Unlike bound phenols, free phenols are available to interact with other molecules.

The phenolic components of apples have been linked with inhibition of colon cancer in vitro (Eberhardt et al., 2000; Veeriah et al., 2006; Kuntz et al., 1999). Eberhardt et al. (2000) reported that apple extracts (both with and without skin) inhibited the proliferation of CaCo-2 cells in a dose-dependent manner, the inhibitory effect was greater in extracts containing apple skins. Veeriah et al. (2006) found that a flavonoid mixture from apples inhibited the proliferation of HT29 cells at sub-cytotoxic concentrations and beneficially influenced the expression of several key genes associated with the transformation of xenobiotics. Individual flavonoids (several of which have been detected in apples) have been shown to inhibit proliferation of CaCo-2 and HT29 cells and to increase apoptosis (Kuntz et al., 1999). Interestingly, several of the more inhibitory flavonoids used in the study are poor anti-oxidants, suggesting that these phenolics may affect CaCo-2 and HT29 proliferation independently of anti-oxidant mechanisms.

In the present study, we have used Bramley apple waste after juice extraction. This material contains the parts of the apple rich in phenolics (Eberhardt et al., 2000; Veeriah et al., 2006) and may represent a valuable resource of health promoting food ingredients. Despite the known high content of phenolics in apples there has been few systematical studies of the potential anti-cancer activity of apple phenolics. Therefore we investigated the ability of a crude extract of the apple waste to affect a range of colon cancer biomarkers, namely: DNA damage (comet assay), colono- cyte barrier function (trans-epithelial resistance assay), cell cycle progression (DNA content assay) and invasion (matrigel invasion assay) in vitro.

2. Materials and methods

2.1. Reagents and apple waste

All reagents were obtained from Sigma (Poole, UK) unless otherwise stated. Bramley apple waste was provided by MacNeice Bros Limited (Portadown, UK). The apple waste consisted of material leftover after juice extraction.

2.2. Extraction of phenolics from apple waste

The insoluble fraction of phenolics from 50 g of apple waste was extracted using a series of homogenisation and filtration steps. Fifty grams of apple waste was macerated in blender (1 min high power, 2 cycles, Waring UK) filtered under vacuum and the extract concentrated to 25 ml by rotary evaporation. Hydrolysis of the apple waste was accomplished by the addition of 15 ml of 5 M sodium hydroxide for 2 h under nitrogen, followed by neutralisation with hydrochloric acid. The aqueous phase was extracted six times with ethyl acetate (15 ml per extraction). The pH of the residual aqueous phase, after ethyl acetate extraction, was adjusted to 7 and applied to a solid phase extraction (SPE) column (Waters). Elution of phenolics from the column was performed in two steps by initially eluting with 20% methanol and concentrated in a rotary evaporator and the concentrate was extracted with ethyl acetate (2x). The SPE columns were then eluted (2x) with ethyl acetate as mobile phase. The above ethyl acetate fractions were combined and concentrated in a rotary evaporator. An aliquot of this extract was used for phenolic content analysis and the remainder of the same was evaporated to dryness and resuspended in 5 ml of water and stored at –70 °C prior to use for in vitro bioassays.

2.3. Colorimetric determination of total phenolic content

The total phenolic contents of the apple peel samples were estimated by using ethanol extracts (0.5 ml) of a known dilution of apple peel extract were added to a test tube containing deionised water and 0.25 ml. Folin-Ciocalteu Reagent (0.25 ml) and the solution was allowed to react for 5 min and followed by addition of 1.5 ml of 7% sodium carbonate solution was aliquoted into the test tubes, and the mixture was diluted to 5 ml with deionised water. The colour developed for 60 min, and the absorbance was measured at 760 nm using a spectrophotometer (Pye-Unicam), calibrated into a standard curve of gallic acid concentrations and expressed as mg gallic acid equivalents/g ± SD fresh apple peels for the duplicate extracts.

2.4. Colorimetric determination of flavonoid content

The flavonoid contents of the apple peel samples were measured by colorimetric method in which 0.2 ml of a known dilution of ethanol extracts of apple peel waste was added to a test tube containing 1.6 ml of distilled water and mixed thoroughly and to the same 0.2 ml of 5% sodium nitrate solution was added and allowed to stand for 3 min. 0.5 ml of 10% aluminium chloride was added. After 10 min, 0.5 ml of 1 M sodium carbonate was added and the volume adjusted to 5 ml using distilled water. The absorbance of the mixture at 510 nm was measured immediately in a Pye-Unicam spectrophotometer and compared to a standard curve of catechin concentrations. The flavonoid content was expressed as mg catechin equivalents/g fresh apple peels for duplicate extracts.
2.5. Colorimetric determination of anthocyanin content

Monomeric anthocyanin content of the apple peels was measured using a spectrophotometric pH differential protocol. The apple peel acetone extracts were mixed thoroughly with 0.025 M potassium chloride (pH 1) buffer yielding extract:buffer 1:3 or 1:8 ratio. The absorbance of the mixture was then measured at 515 and 700 nm against – distilled water blank. The apple peel extracts were then combined similarly with sodium acetate buffer pH 4.5, and the absorbance of these solutions was measured at the same wavelengths. The anthocyanin content was calculated as follows:

\[
\text{Total monomeric anthocyanins (mg/100 g) fresh apple waste = } A \times \frac{M_w}{1000} \times \frac{q}{C_w}
\]

where \( A \) is absorbance = \( (A_{515} - A_{700}) \times 10^4 - (A_{320} - A_{700}) \times 10^4 \) \( M_w \) is molecular weight for cyanidin 3-glucoside = 449.2; \( q \) is the molar absorptivity of cyanidin 3-glucoside = 26,900; and \( C_w \) is the concentration of the buffer in mg/ml. Anthocyanin content was expressed as mg cyanidin 3-glucoside equivalents/g fresh apple peel for the duplicate extracts.

2.6. Reverse phase HPLC-UV diode array detection of individual flavonoids in apple waste

For individual flavonoids, the extract was passed through solid phase extraction kit (Waters) and subjected to reverse phase (RP)-HPLC. The diode array detection and the UV spectra established for eluting peaks via authentic spectra held in a software database of library of flavonoids. The identity of the individual flavonoids contained in the peak elutions were confirmed by their unique M+ ion peak mass spectra of their corresponding methyl ether derivatives via GC–MS (Rao and Cooper, 1995). Trace metabolites in the extracts were ascertained by separation on a paper or HP-thin layer chromatography before confirmation of their identity via capillary zone electrophoresis coupled with GC–MS (Cooper et al., 1999).

2.7. Cell lines and cell culture

Our colonic model system utilised established cell models of: genotoxicity (HT29), invasion and metastatic potential (HT115), and barrier function (CaCo-2) (Glei et al., 2005; Burns and Rowland, 2004; Parr et al., 2000; Jiang et al., 1995; Zolotarevsky et al., 2002). The HT29, HT115, and CaCo-2 cell lines were obtained from the European Collection of Cell Cultures (Sigma, Poole, UK). Cell culture media and supplements were obtained from Gibco (Paisley, UK) unless otherwise stated. The HT29 and HT115 cells were grown in DMEM supplemented with 1% penicillin–streptomycin, 2 mM L-glutamine (HT115 only) and foetal bovine serum (10% HT29, 15% HT115), with the media refreshed every 2 days. The CaCo-2 cells were grown in MEM supplemented with 2 mM L-glutamine, 1% penicillin–streptomycin, and 10% foetal bovine serum, with the media refreshed every 2 days.

2.8. Cell viability assay

HT115 cells were seeded at a density of 3 × 10^5 cells per ml for 24 h before treatment with each apple extract. After exposure, a single-cell suspension of 5 × 10^5 cells per ml was prepared for each treatment. This suspension was centrifuged at 1200 rpm for 3 min, the supernatant discarded and the pellet resuspended in 200 μl of ice cold PBS and 2 ml of 70% ethanol/30% PBS. The cells were incubated on ice for 30 min, centrifuged at 1200 rpm for 3 min, the supernatant carefully discarded, and the cells resuspended in 800 μl of ice cold PBS, 100 μl of RNase A (1 mg/ml Sigma), and 100 μl of propidium iodide (400 mg/ml Sigma). The cells were then incubated at 37 °C, 5% CO₂ for 30 min before being analysed on a FACSCalibur flow cytometer (Becton Dickinson, UK) and the fluorescence emission spectra of propidium iodide was collected at 585 nm, using CellQuest Software (Becton Dickinson). Subsequently, these emission spectra were analysed for DNA content using WinMDI software (J. Trotter, Scripps Inst.). The experiment was performed as triplicate independent experiments.

2.10. Trans-epithelial resistance assay

The effect of a series of apple extract dilutions, 0.02, 0.05 and 0.1 (% extract in media) on trans-epithelial resistance in CaCo-2 cells. Briefly, 2.5 × 10^5 CaCo-2 cells were seeded in 6-well plates with Transwell inserts (0.1% rat tail collagen coated polyethylene terephthalate membranes; BD Bioscience, UK). The culture medium was replaced (apical 5.1.5 ml, basal side 5.2.5 ml) every other day for 14 days. Cells were maintained at 37 °C for 14 days. From days 11–14, the integrity of the monolayer was evaluated by measuring the trans-epithelial resistance (TER) (expressed as Ω/cm²) using an EVOM epithelial voltometer (World Precision Instruments Ltd., Aston, UK). Once the TER values had stabilised, the inserts were ready for experimentation. The TER of the CaCo-2 cell monolayers were measured at zero, 24 and 48 h after the addition of apple extracts to the apical compartment. Quadruplicated measurements of each concentration were taken for each experiment and each experiment was repeated in triplicate.

2.11. Cell cycle analysis

The DNA content of HT115 cells treated with a range of apple extracts, 0.01%, 0.02%, 0.05% and 0.1%, for 24 h was determined using propidium iodide staining and measured using flow cytometry as described previously (Omerod, 2000).

Briefly, HT115 cells were seeded at a density of 3 × 10^5 cells per ml for 24 h before treatment with each apple extract. After exposure, a single-cell suspension of 5 × 10^5 cells per ml was prepared for each treatment. This suspension was centrifuged at 1200 rpm for 3 min, the supernatant discarded and the pellet resuspended in 200 μl of ice cold PBS and 2 ml of 70% ethanol/30% PBS. The cells were incubated on ice for 30 min, centrifuged at 1200 rpm for 3 min, the supernatant carefully discarded, and the cells resuspended in 800 μl of ice cold PBS, 100 μl of RNase A (1 mg/ml Sigma), and 100 μl of propidium iodide (400 mg/ml Sigma). The cells were then incubated at 37 °C, 5% CO₂ for 30 min before being analysed on a FACSCalibur flow cytometer (Becton Dickinson, UK) and the fluorescence emission spectra of propidium iodide was collected at 585 nm, using CellQuest Software (Becton Dickinson). Subsequently, these emission spectra were analysed for DNA content using WinMDI software (J. Trotter, Scripps Inst.). The experiment was performed as triplicate independent experiments.

2.12. Invasion assay

The effect of a series of apple extract dilutions, as used in the cell cycle assay, on the invasion of the HT115 cell line was determined using the Matrigel invasion assay (MIA) (BD Bioscience, UK). The assay was performed both with 24 h of pre-treatment with the extracts and without any pre-treatment. Freshly prepared extract solution was used in invasion assays.

Briefly, 6-well Biocoat Matrigel invasion chambers (inserts) (BD BioScience) were rehydrated with the addition of 2 ml warm serum-free culture medium for 2 h. In parallel to, but separate from insert rehydration, 2 ml of MRC5 cell suspension (4 × 10^5 cells/well) were seeded in the plate and were incubated at 37 °C for 2 h. The media was removed carefully from the plate eliminating any unattached cells in the process and replaced with 2 ml of Ham’s F12 containing 10% FBS per well. Upon completion of insert rehydration, the inserts were transferred to the plate containing the MRC5 cells. The media was removed from the inserts and replaced with 2 ml of PC-3 cell suspension (2 × 10^5 cells, serum free F12) in the presence or absence of apple extract inserts containing cells alone served as a control. Plates were incubated for 24 h. After incubation, medium was removed and cells on both sides of the membrane were fixed in 70% ethanol for 30 min and then stained with hematoxylin. Using a
cotton bud, the non-invasive cells were removed from half of the surface of the insert by “scrubbing”. This process was repeated for the invasive cells on the other side of the insert. The numbers of invasive and non-invasive cells were then counted in five random fields of the insert and the percentage invasion was calculated. An estimate of total cell numbers was also taken by adding the mean number of invasive and non-invasive cells counted on the inserts. The treatments were carried out in duplicate for each experiment, with each experiment repeated independently three times.

2.13. Statistical analyses

All experiments were analysed using SPSS (version 12.0) software. The data was tested normality using the Kolomogorov–Smirnoff test. Normal data was tested for significance using the student’s t-test or one-way ANOVA (LSD or Games–Howell post-hoc tests) as appropriate. Non-normal data was analysed using the Kruskal–Wallis and Mann–Whitney U tests. The significance level taken was *p* < 0.05.

3. Results

3.1. Cell viability assay

No significant differences in viability were observed between the untreated cells and the extract-treated cells, with the level of viability for the extract-treated cells >95%

3.2. Comet assay

When HT29 cells were pre-incubated with 0.2% apple extract for 24 h, DNA damage induced by subsequent exposure to H$_2$O$_2$ challenge was significantly reduced by 69.5% (*p* = 0.02). Lower concentrations of extract however were ineffective. The apple extract alone did not significantly affect DNA damage (data not shown).

3.3. Trans-epithelial resistance assay

The TER assay found that the positive control (DCA) significantly decreased epithelial resistance after 24 and 48 h (−104 and −221 Ω/cm$^2$ respectively, untreated cells value of zero, *p* < 0.05). After 24 h both the 0.1% and 0.05% extracts significantly increased resistance (+36 and +14 Ω/cm$^2$, respectively, *p* < 0.05). After 48 h the increased resistance of the 0.01% and 0.05% extracts was more pronounced that at 24 h (both +63 Ω/cm$^2$, *p* < 0.05). The 0.02% extract had no effect at either time-point.

3.4. Cell cycle analysis

No significant cell cycle profile alterations were measured for the concentrations used.

3.5. Invasion assay

When HT115 cells were incubated on matrigel-coated membrane 13.6 ± 0.5% of cells migrated through the membrane in 24 h. Addition of the apple phenolic extract resulted in a dose-dependent inhibition of invasion ($r^2 = 0.97$, *p* = 0.038) that reached significance at 0.05% and 0.1% concentrations (Fig. 1). The inhibitory effect was greater when the cells were incubated for 24 h prior to the invasion assay. Under these conditions all concentrations tested were significantly inhibitory and at 0.1% the

![Fig. 1. The effect of apple extracts on the invasion of HT115 cells. Cells were either treated during the assay or pre-treated with extracts for 24 h prior to assay. Data are expressed relative to the control as the mean (±standard error of the mean) of triplicate independent experiments. The percentage invasion of the control cells was 13.6 ± 0.5%. Significant differences (*p* < 0.05) between the control and treatments are indicated by *. (SPSS, one-way ANOVA with LSD test.)](attachment:Fig_1.png)
Table 1
Flavonoids present in fresh Bramley apple waste (peel) extracts (micrograms per gram of fresh weight)

<table>
<thead>
<tr>
<th>Flavonoid type/flavonoid</th>
<th>Quantity (% of total and mg/g of fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavan-3-ols</td>
<td>32%</td>
</tr>
<tr>
<td>Catechin</td>
<td>780</td>
</tr>
<tr>
<td>Procyanidin B</td>
<td>1786</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>2550</td>
</tr>
<tr>
<td>Unknown Procyanidin</td>
<td>10</td>
</tr>
<tr>
<td>Phloretin glycosides</td>
<td>24%</td>
</tr>
<tr>
<td>Dihydrochalcones</td>
<td>550</td>
</tr>
<tr>
<td>Dihydroflavanones</td>
<td>245</td>
</tr>
<tr>
<td>Phloretin xyloglycoside</td>
<td>430</td>
</tr>
<tr>
<td>Phloridzin</td>
<td>2470</td>
</tr>
<tr>
<td>Quercetin glycosides</td>
<td>30%</td>
</tr>
<tr>
<td>Rutin</td>
<td>2025</td>
</tr>
<tr>
<td>Isoquercitrin</td>
<td>655</td>
</tr>
<tr>
<td>Quercetin glycoside</td>
<td>1252</td>
</tr>
<tr>
<td>Avicularin</td>
<td>2386</td>
</tr>
<tr>
<td>Quercetin</td>
<td>987</td>
</tr>
<tr>
<td>Cyanidin glycosides</td>
<td>Traces</td>
</tr>
<tr>
<td>Ideain</td>
<td>12</td>
</tr>
<tr>
<td>Hydroxycinnamic acids</td>
<td>10%</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>2318</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>213</td>
</tr>
<tr>
<td>Other unidentified flavonoids</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

The type of flavonoids present in the extracts is shown in bold and their net concentrations as a fraction of total phenols is given in a percentage indicated in parenthesis. The quantification of total phenolic contents (6.9–11.5 mg/g of fresh apple peel waste) and net flavonoids comprising each class of flavonoids has been quantified as a measure of catechin, phlorizin, rutin or p-coumaric acid units initially by colorimetric methods described in materials and methods section. The individual flavonoids were quantified by a combination of techniques (Rao and Cooper, 1995; Cooper et al., 1999).

3.6. Phenolic content analysis

The phenolic content analysis of the apple extract used detected several phenolic compounds associated with beneficial anti-cancer effects, as shown in Table 1.

4. Discussion and conclusions

Our study has demonstrated that the phenolic compounds extracted from apples beneficially modulated three risk biomarkers of colorectal cancer in vitro without any cytotoxic effects. DNA damage was decreased (associated with tumour initiation), colonic barrier function was enhanced (associated with decreasing tumour promotion) and invasive potential was reduced (associated with reduced tumour metastatic potential). Pre-treatment with apple phenolics for twenty-four hours markedly enhanced the anti-invasive effects of the extract. It is to our knowledge the first study to investigate the effects of apple phenolics on the invasion and barrier function of colon cancer cell lines.

Our finding that apple phenolics can protect against DNA damage is in agreement with previous studies highlighting the potent anti-oxidant properties of these compounds (Lee et al., 2003; Sun et al., 2002; Eberhardt et al., 2000). Only the 0.2% apple extract significantly inhibited H2O2-induced damage in HT29 cells (p = 0.02). A significant increase in the barrier function of CaCo-2 cells was measured at 24 and 48 h for both the 0.1% and 0.05% extracts (p < 0.05) indicated by increased resistance measured by the TER assay. A significant decrease in the invasion of HT115 cells was found for the 0.05% and 0.1% extracts after 24 h (p < 0.05). After 24 h of pre-treatment, prior to the 24 h required for the invasion assay, all concentrations used inhibited invasion (p < 0.05). The apple extract had no effects on the cell cycle of HT115 cells (p < 0.05). However, the effect of the 0.02% extract on the sub-G0 fraction of the cell cycle approached significance (p = 0.06). An increase in the sub-G0 population may imply an increased level of apoptosis. This was not a dose-dependent effect so its relevance is unclear. These data suggest that whilst the higher concentrations used were effective after only 24 h; prolonged exposure to lower concentrations was also effective.

Chemical analysis of the extract used indicated that it contained bioactive compounds that have been linked with protective effects both in colorectal and other cancers (van der Sluis et al., 1997; Ebeler et al., 2002; Weyant et al., 2001; Kassai et al., 2000; van der Woude et al., 2003; Veeriah et al., 2006; Kuntz et al., 1999; Peng and Kuo, 2001) to varying degrees. At this stage, it is difficult to assess the potency of individual compounds detected to such bioactivities straightaway. Moreso, the crude extract we used might contain several unknown metabolites which either singly or synergistically in conjunction with specific compounds may have attributed to the reported bioactivity. However, as we used a crude extract, we cannot attribute the reported effects to specific compounds. It is likely that interactions between the various phenolic components of the extract contribute to the overall effects reported and future work involving separation, purification and one to one bioassays of known and unknown metabolite fractions may reveal the true nature and extent of their potency for anti-cancer effects observed in vitro.

Our findings are in agreement with related studies using apple juice extracts. For example, Schaefer et al. (2006) reported a decrease in DNA damage in HT29 and CaCo-2 cells treated with an apple juice/pomace extract. Additionally, two of the major components identified in their extract, quercetin and phloretin, were detected in our apple waste extract. Among them, their glycosides together with cyanidin glycosides are only found in apple peels and generally absent in the flesh of most of the cider apple varieties (van der Sluis et al., 2001).
We have shown that a crude mixture of apple phenolics can affect three biomarkers of colon cancer risk in vitro. In terms of the concentration required, the effects of the extract appeared to be more potent in the bioassays that modelled the later stages of carcinogenesis namely TER (tumour promotion) and matrigel invasion (tumour metastasis). Protection against DNA damage may also reduce the acquisition of mutations leading to tumourigenic phenotype or delay the rate of aggressive mutations. Increased barrier function can potentially prevent abnormal cellular interactions with the surrounding extracellular environment. Inhibition of invasive restricts the process of metastasis and abnormal extracellular interactions. Therefore, apple consumption may serve to protect against the risk of colon cancer by protecting colonic cells against DNA damage and abnormal extracellular behaviour.

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References


