Effects of plant extracts on antioxidant status and oxidant-induced stress in Caco-2 cells

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Experimental evidence suggests that most herbs and spices possess a wide range of biological and pharmacological activities that may protect tissues against O_2-induced damage. The objectives of the present study were: first, to determine the effects of plant extracts on the viability, membrane integrity, antioxidant status and DNA integrity of Caco-2 cells and second, to investigate the cytoprotective and genoprotective effects of these plant extracts against oxidative stress in Caco-2 cells. The plant extracts examined were rosemary (*Rosmarinus officinalis* L.), oregano (*Origanum vulgare* L.), sage (*Salvia officinalis* L.) and echinacea (*Echinacea purpurea* L.). Cell membrane integrity was assessed by the lactate dehydrogenase release assay. Viability was determined by the neutral red uptake assay (NRUA) and the concentration of compound that resulted in 50 % cell death (IC_{50}) was calculated. Antioxidant status of the cells was assessed by measuring GSH content, catalase activity and superoxide dismutase activity. To examine their cytoprotective and genoprotective effects, Caco-2 cells were pre-treated with each plant extract for 24 h followed by exposure to H_2O_2. DNA damage was assessed by the comet assay and cell injury was determined by the NRUA. Rosemary was the most toxic (IC_{50} 123 μg/ml) and echinacea the least toxic (IC_{50} 1421 μg/ml). Sage was the only plant extract to affect the antioxidant status of the cells by increasing GSH content. Sage, oregano and rosemary protected against H_2O_2-induced DNA damage (olive tail moment and percentage tail DNA), whereas protection against H_2O_2-induced cytotoxicity was afforded by sage only.

Herbs: Antioxidants: DNA damage: Comet assay: Caco-2 cells

Oxidative stress and reactive oxygen species-mediated cell damage have been implicated in the development of various human chronic diseases such as Crohn's disease, CVD, certain cancers and a number of neurodegenerative diseases (Babbs, 1992; Halliwell & Gutteridge, 1999; Parthasarathy *et al.* 1999; Sohal *et al.* 2002). At the cellular level, subjecting cells to oxidative stress can result in severe metabolic dysfunction, including lipid peroxidation, protein oxidation, membrane disruption and DNA damage (Halliwell & Gutteridge, 1999).

Oxidative damage can also have undesirable effects on foods such as the production of rancid odours and flavours which can reduce the shelf-life, nutritional quality and safety of food products (Zainol *et al.* 2003; Chanwitheesuk *et al.* 2005). In order to lower the risk of oxidative deterioration, synthetic antioxidants have been added to many food products. However, due to safety issues and consumer demand, there has been considerable interest in replacing synthetic antioxidants with natural plant-based alternatives. Therefore, an increasing number of plant-derived antioxidant preparations are commercially available for use in food applications (Aruoma *et al.* 1996; Mielnik *et al.* 2003).

Over the past few decades, research has focused on the health effects of phytochemicals and plant-derived extracts. The Lamiaceae family is a large group of plant species that contain substantial amounts of phenolic compounds (Moreno *et al.* 2006). Popular varieties from this family include the culinary herbs: rosemary (*Rosmarinus officinalis* L.), oregano (*Origanum vulgare* L.) and sage (*Salvia officinalis* L.). Experimental evidence suggests that most herbs and spices, especially those of the Lamiaceae family, possess a wide range of biological and pharmacological activities that may protect tissues against O_2-induced damage and therefore lower the risk of human chronic diseases (Craig, 1999; Kris-Etherton *et al.* 2002; Bozin *et al.* 2006).

Natural phytochemicals present at low levels in fruit, vegetables, herbs and spices offer many health benefits, but these compounds may not be effective or safe when consumed at higher does (Liu, 2003). If herbal use is to be extended to applications that require high doses, the increased exposure to human consumers is a matter of concern (Aydin *et al.* 2005). The objective of the present study, therefore, was to investigate the toxicity and biological activity of rosemary, oregano, sage and echinacea extracts using a human colon carcinoma cell line, Caco-2 cells, as a model system. The paper presents the effects of rosemary, oregano, sage and echinacea extracts on the viability, membrane integrity, antioxidant status and DNA integrity of Caco-2 cells. In the present study, the four plant extracts were also investigated for their potential cytoprotective and genoprotective effects against H_2O_2-induced stress in Caco-2 cells.

Materials and methods

Materials

All chemicals and cell culture reagents were obtained from Sigma Chemical Co. (Poole, Dorset, UK) unless otherwise stated. Rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), sage (*Salvia officinalis*) and echinacea (*Echinacea purpurea* (L.), Moench–Asteraceae family) extracts were purchased from Guinness Chemicals (Portlaoise, Co. Laois, Republic of Ireland). Suppliers provided the following information based on HPLC analysis: No information was available on the oregano extract. Rosemary extract contained rosmarinic acid (7 %, w/v). The key compounds detected in the sage extract were rosmarinic acid (7·03 %, w/v), flavones (3·21 %, w/v) and flavanones (5·13 %, w/v). Echinacea extract contained various caffeic acid compounds (8·35 % total, w/v).

Total phenol content

Total phenolic content of the extracts was determined by employing the methods given in the literature involving Folin–Ciocalteu reagent and gallic acid as standard (Singleton & Rossi, 1965).

Cell culture

Human Caucasian colon adenocarcinoma Caco-2 cells were purchased from the European Collection of Animal Cell Cultures (Salisbury, Wilts, UK). Cells were cultured in an atmosphere of CO_2–air (5:95, v/v; O_2 partial pressure of 19·998 kPa) at 37°C. Caco-2 cells were grown in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum (10 %, v/v) and non-essential amino acids (1 %, v/v). Cells were maintained in the absence of antibiotics. For all experiments, Caco-2 cells were seeded at a density of 3×10^4 cells per cm^2 in cell culture plates.

Incubation of Caco-2 cells with plant extracts or oxidants

After 24 h growth in the appropriate culture plates, growth media was replaced with growth medium containing reduced fetal bovine serum (5 %, v/v) and non-essential amino acids (1 %, v/v) in the presence or absence of the herb extracts, except for the lactate dehydrogenase (LDH) release assay where 2·5 % (v/v) fetal bovine serum was used. Rosemary, oregano, sage and echinacea extracts were dissolved in non-supplemented Dulbecco's modified Eagle's medium. Control cultures were exposed to the equivalent concentration of carrier and were found not to significantly differ from cells grown in media only. H_2O_2 was dissolved in ice-cold PBS and treatments with the oxidant were in the absence of serum.

For the determination of cell viability, concentration resulting in 50 % cell death (IC_{50}) values and cell membrane integrity, Caco-2 cells were supplemented with increasing concentrations (5–1000 μg/ml) of rosemary, oregano, sage or echinacea extracts for 24 h. For subsequent assays, the concentration of each plant extract that corresponded to greater than 90 % cell viability was selected, namely: 15 μg/ml rosemary, 60 μg/ml oregano, 60 μg/ml sage and 250 μg/ml echinacea. Cell viability was also determined in Caco-2 cells exposed to H_2O_2 (50 μM for 30 min) and (100–500 μM for 2 h). To determine protection against oxidant-induced DNA damage, cells were supplemented with or without the plant extracts for 24 h followed by exposure to 50 μM-H_2O_2 for 30 min at 37°C. To investigate protection against oxidant-induced cytotoxicity, Caco-2 cells were supplemented in the presence or absence of each plant extract for 24 h followed with or without exposure to H_2O_2 (100–500 μM) for 2 h at 37°C.

Cell membrane integrity (lactate dehydrogenase release assay)

Media was collected from all test wells of a ninety-six-well plate. LDH leakage into the growth media and total LDH (LDH leakage plus LDH inside cells) were measured using an *in vitro* lactate dehydrogenase release assay kit (Biogenesis, Poole, Dorset, UK). LDH release was expressed as a percentage of total LDH.

Cell viability (neutral red uptake assay)

The neutral red uptake assay, by the method of Babich & Borenfreund (1992), was used to assess cell viability. Neutral red dye was prepared as a 0·4 % (w/v) stock solution, which was diluted 1/100 using Dulbecco's modified Eagle's medium and pre-incubated overnight at 37°C. After 3 h incubation with the dye (40 μg/ml) at 37°C, cells were quickly washed with a fixative (1 % $CaCl_2$, 0·5 % formaldehyde) and then solubilised. Absorbance was read at 540 nm on a microtitre plate reader. The IC_{50} value for each plant extract (concentration of compound that reduced cell viablity by 50 %) was calculated using the data obtained from the neutral red uptake assay and GraphPad Prism software version 4.0 (GraphPad Inc., San Diego, CA, USA).

Reduced glutathione content

GSH content was determined by the method of Hissin & Hilf (1976). Perchloric acid (15 %, v/v) was added to the cell sonicates and samples were centrifuged at 14 000 rpm for 30 min at 4°C. The final GSH assay mixture contained 100 μl sample, 1·8 ml phosphate–EDTA buffer (pH 8) and 100 μl σ-phthaldialdehyde (1 mg/ml). Fluorescence was detected at 430 nm following excitation at 360 nm. GSH content was expressed relative to the protein content, as determined by the bicinchoninic acid method (Smith *et al.* 1985).

Antioxidant enzyme activity assays

Catalase activity was determined using a modification of the method of Baudhuin *et al.* (1964), where any remaining H_2O_2 is determined as a yellow 'peroxy titanium sulfate'. One unit of catalase activity is defined as the amount of catalase required to decompose 1 μmol H_2O_2 per min at pH 7·5 and 25°C. Superoxide dismutase activity was measured by the method of McCord & Fridovich (1969). One unit of superoxide dismutase activity is defined as the amount of superoxide dismutase required to inhibit the maximum rate of cytochrome C reduction by 50 %. Enzyme activities were expressed as units of enzyme activity per mg protein (Smith *et al.* 1985).

Alkaline single cell gel electrophoresis (comet) assay

DNA damage was assessed using the comet assay by the method of Tice *et al.* (1990). Briefly, treated and non-treated

cells were gently scraped and embedded in low-melting-point agarose on coded microscope slides. Samples were then placed in cold lysis solution (2·5 M-NaCl, 100 mM-EDTA, 10 mM-tri(hydroxymethyl)-aminomethane, 1 % sodium sarcosinate (pH 10), with fresh 1 % Triton® X-100 and 10 % dimethyl sulfoxide) for 1·5 h at 4°C. Slides were aligned in a horizontal gel electrophoresis tank (Horizon® 20·25; GIBCO BRL Life Technologies, Gaithersburg, MD, USA) which was filled with fresh electrophoresis solution (1 mM-EDTA, 300 mM-NaOH; pH 13). Slides were allowed to sit in this buffer for 30 min. Electrophoresis was conducted at 1 V/cm for 25 min at 4°C. After electrophoresis, the slides were washed three times with neutralising buffer and stained with ethidium bromide (20 µg/ml). Fifty nuclei per slide were analysed using computer-assisted image analysis (Komet 4; Kinetic Imaging, Liverpool, UK). The extent of DNA damage was expressed in two ways: (i) the mean percentage of tail DNA, reflecting the proportion of DNA that has migrated from the head and (ii) olive tail moment (OTM), which represents the product of the tail length and the percentage DNA in the tail.

Statistical analysis

Results are presented as mean values with their standard errors. Data were analysed by one-way ANOVA followed by Dunnett's test. The level of statistical significance was taken at $P < 0.05$ or $P < 0.01$.

Results

Total phenolic content of plant extracts

The total phenolic content of the four plant extracts was as follows: rosemary, 207 mg gallic acid equivalents (GAE)/g; sage, 192 mg GAE/g; oregano, 78 mg GAE/g; echinacea, 58 mg GAE/g.

Effects of plant extracts on the membrane integrity of Caco-2 cells

Caco-2 cells were grown in the presence or absence of rosemary, oregano, sage or echinacea (5–1000 µg/ml) for a period of 24 h. Following incubation, cell membrane integrity was determined using the LDH release assay. At concentrations ≥ 120 µg/ml, rosemary and sage significantly increased LDH leakage from the cells (Fig. 1). Supplementation of Caco-2 cells with oregano (≥ 500 µg/ml) or echinacea (1000 µg/ml) significantly induced LDH release (Fig. 1). The membrane-toxic effects of the plant extracts were in the order of rosemary > sage > oregano > echinacea.

Effects of plant extracts on cell viability in Caco-2 cells

Caco-2 cells were supplemented with or without increasing concentrations of rosemary, oregano, sage or echinacea (5–1000 µg/ml) for 24 h. Following incubation, the effect of the plant extracts on cell viability was assessed using the neutral red uptake assay. Cell viability in control and carrier control samples was greater than 95 % for all experiments (data not shown). On the addition of rosemary (≥ 30 µg/ml), cell viability was significantly reduced ($P < 0.01$; Fig. 2). The addition of higher concentrations of sage (≥ 120 µg/ml) or oregano (≥ 250 µg/ml) significantly decreased cell viability compared with control cells ($P < 0.01$). Echinacea was toxic only at the highest concentration tested ($P < 0.01$; Fig. 2). The cytotoxic effects of the plant extracts were in the order of rosemary > sage > oregano > echinacea. Using data obtained from the neutral red uptake assay, the IC_{50} value for each plant extract was determined. Rosemary had an IC_{50} value of 123 µg/ml, sage had an IC_{50} value of 214 µg/ml whereas oregano had a value of 318 µg/ml. Because the highest concentration of echinacea resulted in approximately 64 % cell viability ($P < 0.01$), the predicted IC_{50} value was
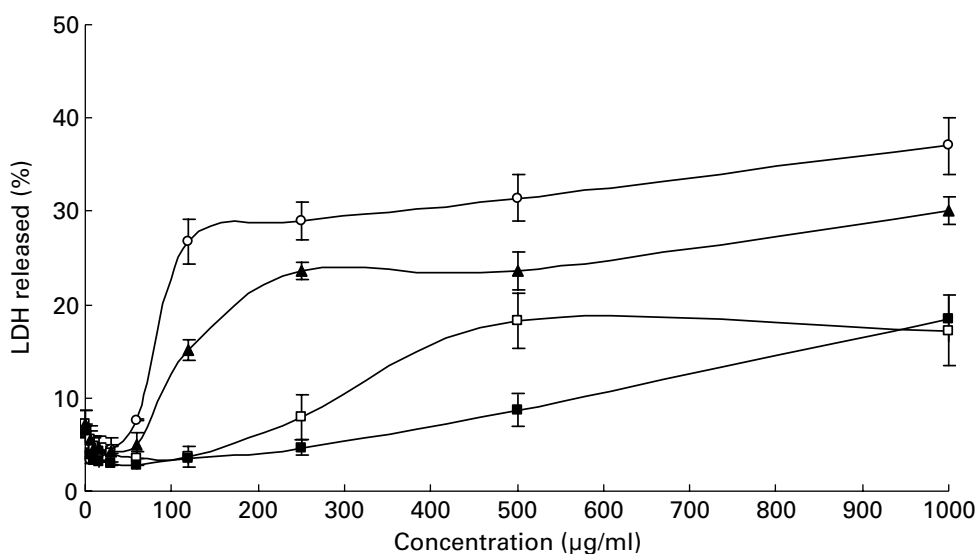

Fig. 1. Cell membrane integrity of Caco-2 cells following supplementation with or without increasing concentrations (5–1000 µg/ml) of rosemary (-○-), oregano (-□-), sage (-▲-) or echinacea (-■-) extracts for 24 h. Cell membrane integrity was assessed using the lactate dehydrogenase release assay (see p. 322). Data are the means of four independent experiments, with standard errors represented by vertical bars.
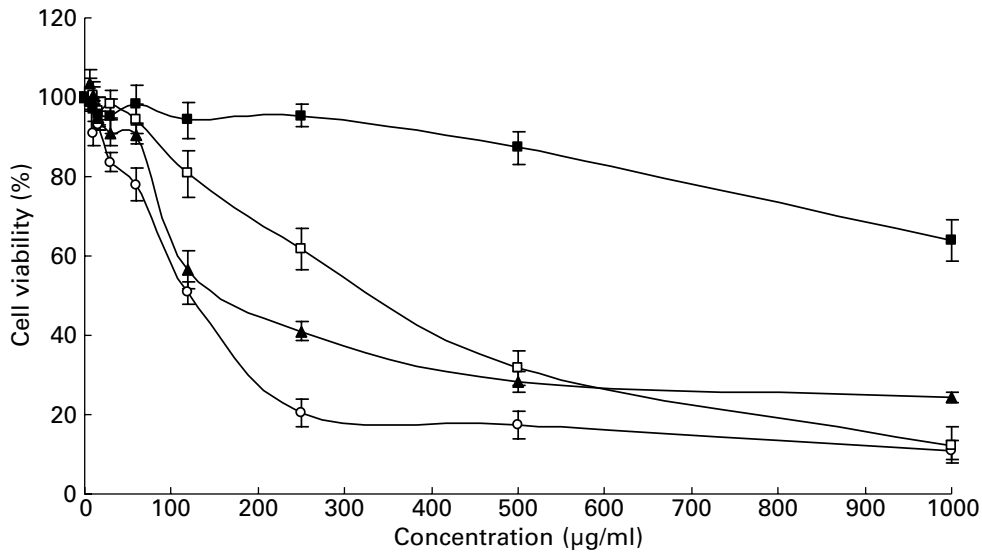
Fig. 2. Viability of Caco-2 cells following supplementation with increasing concentrations (5–1000 μg/ml) of rosemary (-○-), oregano (-□-), sage (-▲-) or echinacea (-■-) extracts for 24 h. Cell viability was determined using the neutral red uptake assay (see p. 322). Data are the means of four independent experiments, with standard errors represented by vertical bars.

1421 μg/ml. Concentrations below the IC$_{50}$ value that corresponded to greater than 90 % cell viability were selected for use in subsequent assays. Exposing Caco-2 cells to H$_2$O$_2$ (50 μM for 30 min) in the absence of serum did not affect cell viability (data not shown).

Effects of plant extracts on antioxidant status of Caco-2 cells

Caco-2 cells were supplemented with or without rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) for a period of 24 h. Following incubation, GSH content, catalase activity and superoxide dismutase activity were determined. Treatment with rosemary, oregano or echinacea had no effect on cellular GSH content whereas

incubation of cells with sage for 24 h significantly increased GSH content ($P < 0.01$) compared with control cells (Fig. 3). There was no significant difference in catalase activity and superoxide dismutase activity between treated and non-treated cells (data not shown).

Effects of plant extracts or hydrogen peroxide on DNA integrity of Caco-2 cells

The potential of the plant extracts to induce single-strand DNA breaks was assessed using the comet assay. Caco-2 cells were supplemented with or without rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) for 24 h. None of the four plant extracts investigated significantly
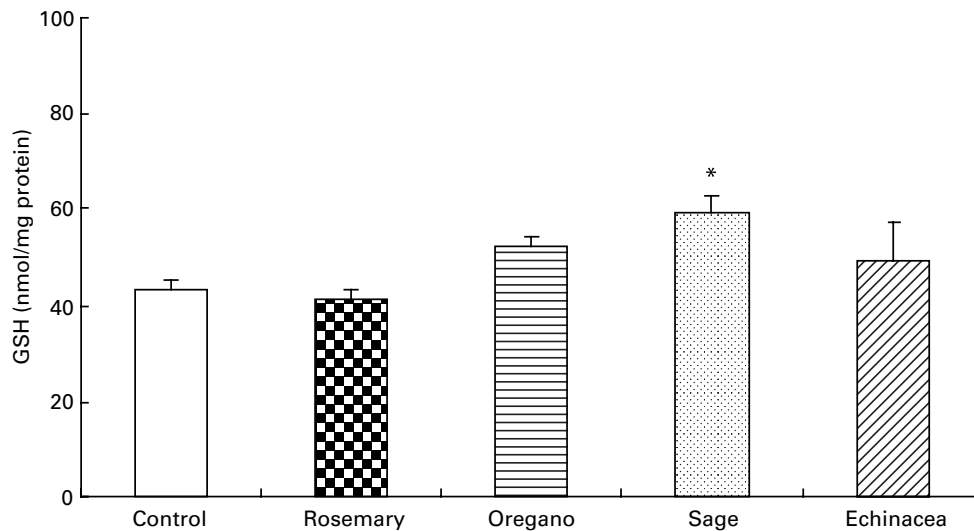

Fig. 3. GSH content of Caco-2 cells following supplementation with or without rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) extracts for 24 h. Data are the means of four independent experiments, with standard errors represented by vertical bars. * Mean value was significantly different from that for control ($P < 0.01$; one-way ANOVA followed by Dunnett's test).

increased the formation of DNA single-strand breaks when compared with control cells (data not shown). Caco-2 cells were exposed to 50 μM-H_2O_2 for 30 min at 37°C. Addition of H_2O_2 to the culture medium significantly increased DNA damage both in terms of percentage tail DNA and OTM ($P<0.01$; Figs. 4 (A) and (B)).

Effects of plant extracts on hydrogen peroxide-induced DNA damage

Caco-2 cells were pre-incubated with or without rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) extracts for 24 h followed by exposure to 50 μM-H_2O_2 for 30 min at 37°C. Similar trends of genoprotection were seen when DNA damage was expressed as either percentage tail DNA or OTM (Figs. 4 (A) and (B)). Pre-treatment with rosemary or oregano significantly decreased H_2O_2-induced DNA damage ($P<0.05$) when compared with cells treated only with H_2O_2 (Figs. 4 (A) and (B)). Supplementation

with sage produced a more significant reduction in H_2O_2-induced DNA damage ($P<0.01$; Figs. 4 (A) and (B)).

Effects of plant extracts on hydrogen peroxide-induced cell injury

Caco-2 cells were pre-incubated with or without rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) extracts for 24 h followed by exposure to increasing concentrations of H_2O_2 (100–500 μM) for 2 h at 37°C. Although treatment with 100 μM-H_2O_2 for 2 h reduced cell viability, the effect was not significant (Table 1). None of the plant extracts modulated this oxidant-induced reduction in cell viability. The addition of higher concentrations of H_2O_2 (250 and 500 μM) significantly decreased ($P<0.01$) the viability of cells compared with control (Table 1). Sage was the only extract to significantly protect against H_2O_2-induced cytotoxicity ($P<0.05$). Although not significant, pre-treatment
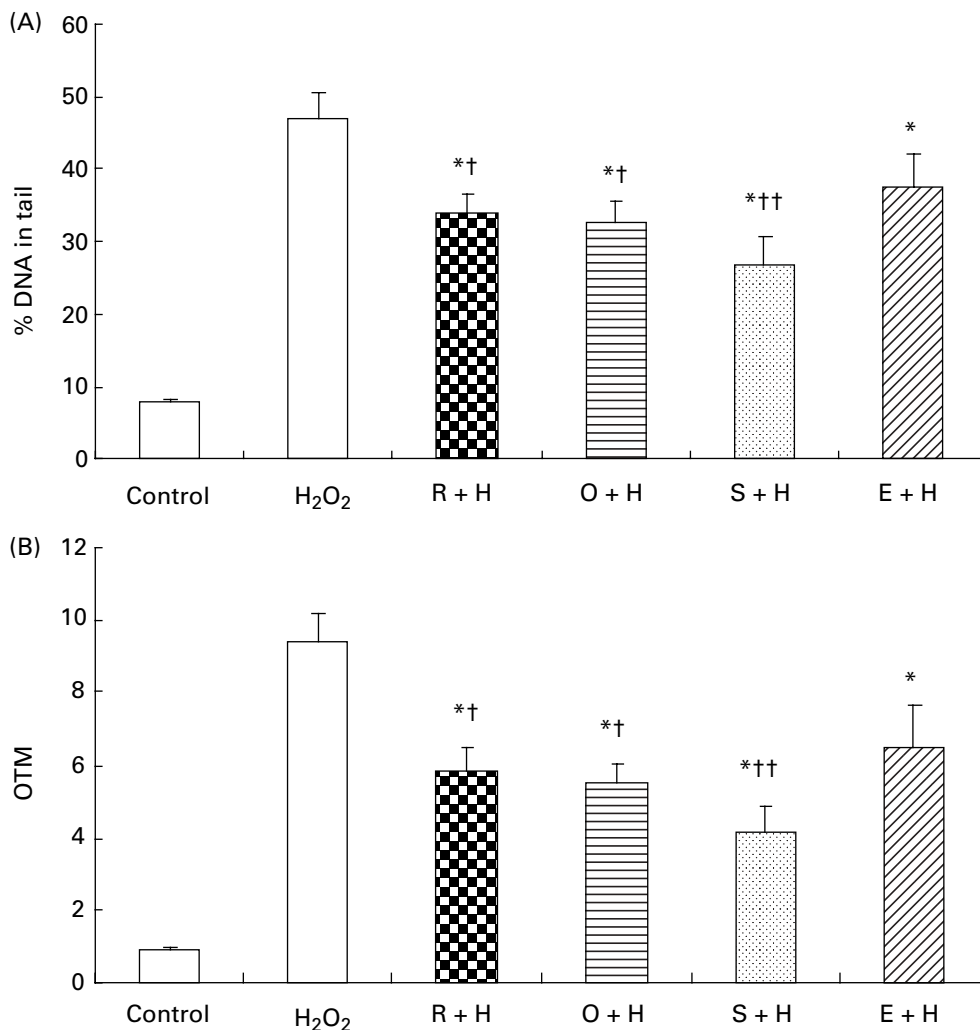

Fig. 4. DNA damage in Caco-2 cells following pre-treatment with or without extracts of rosemary (R; 15 μg/ml), oregano (O; 60 μg/ml), sage (S; 60 μg/ml) or echinacea (E; 250 μg/ml) for 24 h then exposed to 50 μM-H_2O_2 (H) for 30 min at 37°C. DNA damage was assessed by the comet assay (see pp. 322–323). (A) Percentage tail DNA; (B) olive tail moment (OTM), which represents the product of the tail length and the percentage tail DNA. Data are the means of four independent experiments, with standard errors represented by vertical bars. Statistical analysis was by one-way ANOVA followed by Dunnett's test. * Mean value was significantly different from that for control ($P<0.01$). Mean value was significantly different from that for H_2O_2-treated cells: † $P<0.05$, †† $P<0.01$.

Table 1. Viability of Caco-2 cells after pre-treatment with or without plant extracts for 24 h followed by exposure to hydrogen peroxide‡

(Mean values with their standard errors; three individual experiments)

Treatments...	Cell viability (%)§					
	100 μm		250 μm		500 μm	
	Mean	SEM	Mean	SEM	Mean	SEM
H_2O_2	69·6	6·0	27·9*	8·4	18·9*	9·9
Rosemary + H_2O_2	70·6	4·7	29·4*	3·4	35·8*	7·1
Oregano + H_2O_2	62·3	0·7	28·0*	4·1	23·5*	1·6
Sage + H_2O_2	70·9	6·3	55·5*†	4·6	49·6*†	3·6
Echinacea + H_2O_2	80·3	14·4	46·3*	11·0	35·4*	5·9

*Mean value was significantly different from that for control ($P < 0.01$; one-way ANOVA, followed by Dunnett's test).
†Mean value was significantly different from that for H_2O_2-treated cells ($P < 0.05$; one-way ANOVA, followed by Dunnett's test).
‡Caco-2 cells were grown in the presence or absence of rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) extracts for 24 h followed by exposure to increasing concentrations of H_2O_2 for 2 h at 37°C.
§Determined using the neutral red uptake assay (see p. 322). Results are expressed as a percentage of the control (100 %).

with rosemary and echinacea did show a certain degree of cytoprotection against H_2O_2 (Table 1).

Discussion

Fruits, vegetables and herbs contain a wide variety of phytochemicals that possess bioactive properties which may help protect cellular systems from oxidative damage (Liu, 2003). In the present study, the biological effects of four plant extracts, namely rosemary, oregano, sage and echinacea were investigated. While there has been interest in the antioxidant activity of rosemary, oregano and sage and immunomodulatory effects of echinacea, limited information exists on their cytoprotective and genoprotective effects on cells in culture.

Many constituents in rosemary, oregano and sage such as phenolic acids (including rosmarinic and caffeic), phenolic compounds and flavonoids have been reported to exhibit a broad range of biological activities *in vitro* and *in vivo* including antioxidant, anti-inflammatory, anti-microbial, anti-mutagenic, and anti-tumour effects (Aruoma *et al.* 1996; Miura *et al.* 2002; Petersen & Simmonds, 2003; Chorianopoulos *et al.* 2004; Osakabe *et al.* 2004; Aydin *et al.* 2005; Capecka *et al.* 2005). *Echinacea purpurea* has been reported to act as an anti-inflammatory agent and an immunostimulant (Craig, 1999; Linde *et al.* 2006) and is widely consumed for the prevention and treatment of upper respiratory tract infections (Barrett *et al.* 1999, 2002; Hwang *et al.* 2004; Linde *et al.* 2006). However, the exact mechanism(s) of action remain unclear and available evidence from clinical trials on the effectiveness of echinacea has been considered inconsistent (Barrett *et al.* 2002; Linde *et al.* 2006).

To establish the effects of the four plant extracts on membrane integrity in Caco-2 cells, leakage of the membrane-bound enzyme LDH was determined. The order of 'membrane potency' among the four plant extracts correlated well with our cytotoxicity data and IC_{50} values. Supplementation with increasing concentrations of rosemary for 24 h caused cytotoxicity at concentrations ≥ 30 μg/ml in Caco-2 cells. Slameňová *et al.* (2002) determined the viability of Chinese hamster lung

fibroblast V79 cells following supplementation with various concentrations of rosemary for 24 h. Cytotoxicity was reported at concentrations ≥ 15 μg/ml. In the present study both oregano and sage, also members of the Lamiaceae family, affected cell viability in a dose-dependent manner similar to that of rosemary. However, rosemary, having the lowest IC_{50} value of 123 μg/ml, was most toxic of the extracts to Caco-2 cells and echinacea was the least toxic with a predicted IC_{50} of 1421 μg/ml. Carpenter *et al.* (2006) who investigated the effects of various plant extracts on the viability of U937 cells reported that echinacea was the least toxic plant extract of those tested, with the highest IC_{50} value of 9400 μg/ml.

There are many ways to express DNA damage as assessed by the comet assay, and the two most common endpoints reported are OTM and percentage tail DNA. According to Møller (2006), in order to provide feasible comparisons of DNA damage between studies, it is important that common reference endpoints are agreed upon and that researchers provide the opportunity for comparison. Therefore we expressed DNA damage in terms of OTM and percentage tail DNA (Fig. 4). Similar trends were seen when DNA damage was expressed as either of these two endpoints.

It is known that DNA strand breaks occur when cells are exposed to H_2O_2 (Meneghini & Martins, 1993; Kruszewski *et al.* 1994). In the present study, we exposed Caco-2 cells to 50 μM-H_2O_2 for 30 min at 37°C which resulted in significant DNA single-strand-break formation (Figs. 4 (A) and (B)). These findings are in agreement with previous work carried out in our laboratory (Aherne & O'Brien, 1999, 2000). The plant extracts themselves did not induce DNA damage at the concentrations and exposure times tested. Even though oregano extract had less than half the total phenolic content of rosemary or sage, we found that sage, oregano and rosemary, in that order, protected against H_2O_2-induced DNA damage in Caco-2 cells. Research has shown that the antioxidant effectiveness of rosemary, oregano and sage extracts *in vitro* is due to their ability to act as reducing agents and free radical scavengers, as quenchers of singlet O_2 formation and to complex with pro-oxidant metal ions (Miura *et al.* 2002; Dorman *et al.* 2003; Amarowicz *et al.* 2004; Moreno *et al.* 2006; Slameňová *et al.* 2002). Thus the genoprotective effects of the plant extracts could be due, in part, to their free radical-scavenging efficiency and reducing power, as a result of their phenolic and/or non-phenolic constituents.

Sage was the only plant extract to exert a protective effect against H_2O_2-induced cytotoxicity. Therefore, sage may afford this protection against H_2O_2-induced cell injury by mechanisms other than those already mentioned. Chiou & Tzeng (2000) reported that the resistance of K300 cells to menadione-induced cytotoxicity may be due, in part, to the elevated concentrations of GSH in these cells. Therefore, it is possible that sage protected against H_2O_2-induced cytotoxicity by improving the antioxidant status of Caco-2 cells. The present results show that rosemary, oregano and echinacea had no effect on the antioxidant status of Caco-2 cells or on H_2O_2-induced cytotoxicity. However, supplementation with sage enhanced cellular GSH content and protected cells against H_2O_2-induced cytotoxicity. This mechanism could also explain why sage afforded the greatest protection against H_2O_2-induced DNA damage.

Prevention is a more effective strategy than treatment of chronic diseases. Incorporation of herbs into everyday meals may be beneficial, as a diet in which culinary herbs are used generously provides a variety of active phytochemicals that could promote good health by protecting tissues against O_2-induced damage, and preventing the onset of chronic diseases (Craig, 1999). Health benefits resulting from the use of natural plant products rich in bioactive substances has promoted growing interest from pharmaceutical, food and cosmetic industries (Capecka *et al.* 2005). The findings of the present study suggest that the proper use of herb products is safe and may provide some beneficial effects; however, our toxicity data justify concerns regarding the consumption of high doses that are unsafe and possibly harmful to human health.

Acknowledgements

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