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Essiac tea: Scavenging of reactive oxygen species and effects on DNA damage

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Abstract

Essiac, a tea reportedly developed by the Ojibwa tribe of Canada and widely publicized as a homeopathic cancer treatment, is prepared from a mixture of four herbs *Arctium lappa*, *Rumex acetosella*, *Ulmus rubra* and *Rheum officinale*. Each of these herbs has been reported to possess antioxidant and anti-cancer activity. Essiac itself has also been reported to demonstrate anti-cancer activity in vitro, although its effects in vivo are still a matter of debate. We prepared an extract of Essiac tea from a concentration of 25 mg/mL and boiled it for 10 min. From this preparation we used concentrations of 5, 10, 25 and 50% to measure Essiac effects. In this study, we examined the effects of Essiac on free radical scavenging and DNA damage in a non-cellular system, as well as the effects Essiac on lipid peroxidation using the RAW 264.7 cell line. We observed, using electron spin resonance, that Essiac effectively scavenged hydroxyl, up to 84% reduction in radical signal at the 50% tea preparation concentration, and superoxide radicals, up to 82% reduction in radical signal also at the 50% tea preparation concentration, as well as prevented hydroxyl radical-induced DNA damage. In addition, Essiac inhibited hydroxyl radical-induced lipid peroxidation by up to 50% at the 50% tea preparation concentration. These data indicate that Essiac tea possesses potent antioxidant and DNA-protective activity, properties that are common to natural anti-cancer agents. This study may help to explain the mechanisms behind the reported anti-cancer effects of Essiac.

Keywords: Essiac tea; Reactive oxygen species; Lipid peroxidation; ESR; DNA damage

1. Introduction

Essiac is a tea prepared from a mixture of herbs [burdock root (*Arctium lappa*) (Bryson et al., 1978; Rhoads et al., 1984), sheep sorrel (*Rumex acetosella*) (Weber, 2004), slippery elm bark (*Ulmus rubra*) (Choi et al., 2002; Langmead et al., 2002; Brown et al., 2004), and turkey rhubarb (*Rheum officinale*) (Shah et al., 1972; Babu et al., 2003; Suresh et al., 2004)], and has been used in alternative medicine for over 50 years. Burdock root has been shown to have flavonoid-like antioxidants and polyphenols which are also strong antioxidants. Slippery elm bark has been shown to have anti-tumor, anti-inflammatory, and antioxidant activities. Sheep sorrel has demonstrated anti-inflammatory, antioxidant, anti-cancer and even antibacterial properties. The active ingredient in turkey rhubarb, anthraquinones, has been tested experimentally and found to have anti-inflammatory, anti-septic, anti-spasmodic and anti-tumor activities (Tamayo et al., 2000). The phytochemicals found in these ingredients have several possible modes of action which include: antioxidant which protects cells against oxidative damage, interference with DNA replication and antibacterial effects. Although Essiac has been used to treat a variety of conditions as diverse as allergies, hypertension, and osteoporosis, its primary use continues to be the treatment of cancer.

Abbreviations: DMEM, Dulbecco's Modified Eagles Media; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EDTA, ethylenediaminetetraacetic acid; ESR, electron spin resonance; FBS, fetal bovine serum; MDA, malondialdehyde; PBS, phosphate-buffered saline; ROS, reactive oxygen species

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Evidence for the efficacy of Essiac against cancer is mostly anecdotal. To date, only one clinical trial has been conducted with Essiac. The study involved patients suffering from advanced breast cancer and failed to show any benefits of Essiac consumption in those patients (Kaegi, 1998; Boon et al., 2000). Despite a lack of clinical studies reporting efficacy, 72% of the patients taking Essiac reported a positive opinion of the product (Richardson et al., 2000). Flor-Essence, a tea that is similar to Essiac, but contains four additional herbs watercress (*Rorippa microphylla*), blessed thistle (*Carbenia benedictus*), red clover (*Trifolium pretense*), and kelp (*Hexagrammos decagrammus*), did not prevent, but actually promoted DMBA-induced mammary tumor initiation in Sprague–Dawley rats (Bennett et al., 2004).

In vitro, Essiac has been shown to inhibit cell proliferation and induce differentiation in human prostate cancer cell lines (Ottenweller et al., 2004; Tai et al., 2004).

Reactive oxygen species (ROS) have been associated with pathogenic processes including carcinogenesis through direct effects on DNA directly and by acting as a tumor promoter (Kehrer, 1993; Salah et al., 1995; Wiseman and Halliwell, 1996; Vallyathan and Shi, 1997; Kong et al., 2001). Radicals have been demonstrated to be initiators of the oxidative process (Pietraforte et al., 2002), and to be involved in the development of disease (Aust et al., 1993; Stohs, 1995). Catalase, superoxide dismutase, glutathione and uric acid are examples of antioxidants produced by organisms under normal conditions as part of a defense system against ROS-mediated cellular injury. However, if this defense system is challenged or overwhelmed by excessive generation of ROS, redox imbalance or oxidative stress may occur. This can result in damage to the organism (Farber, 1988; Langard, 1990), and disease initiation (Halliwell and Gutteridge, 2000). ROS have also been shown to play an important role in carcinogenesis by damaging DNA and acting as tumor promoters (Wiseman and Halliwell, 1996; Kong et al., 2001).

While Essiac has been indicated as a cancer treatment agent its antioxidant properties have not been well defined. The present study uses radical generating systems for both hydroxyl ($^{\bullet}OH$) and superoxide ($O_2^{\bullet -}$) radicals in order to examine Essiac's ability to scavenge these free radicals. The $^{\bullet}OH$ radical was generated by the Fenton reaction between $FeSO_4$ and H_2O_2 while the $O_2^{\bullet -}$ radical was generated from the reaction of xanthine and xanthine oxidase. RAW 264.7 cells stimulated by Cr(VI) was used as a cellular source of radicals. The cells generated radicals after exposure to Cr(VI) similar to findings in other studies (Leonard et al., 2004) and Essiac was added to measure its effects on radicals produced in this system. Our study also used the Fenton reaction to generate $^{\bullet}OH$ radicals in order to measure lipid peroxidation, DNA damage, and the possible effects of Essiac on these systems. Major questions we wish to address in our study are: (1) What is the effect of Essiac on $^{\bullet}OH$ radicals? (2) What is the effect of Essiac on $O_2^{\bullet -}$ radicals? (3) What is the effect of Essiac on radicals generated in a cellular system? (4) Can Essiac affect lipid peroxidation in a cellular system? (5) What effects does Essiac have on DNA damage caused by free radicals?

2. Materials and methods

2.1. Materials

Essiac was obtained as a kind gift from Mr. Al Hoffman and was prepared as a mixture of four traditionally used herbs. The relative amounts of each were: burdock root (*Arctium lappa*) roots (680 g), sheep sorrel (*Rumex acetosella*) aerial parts (455 g), slippery elm (*Ulmus rubra*) leaves (120 g), and turkey rhubarb (*Rheum officinale*) root (30 g). The tea was prepared by adding Essiac tea powder to distilled water (25 g/L H_2O) and boiling for 10 min. The tea was then left to cool for 4 h followed by another 5 min boil. After overnight storage in a refrigerator one fraction was filtered. Both fractions were stored at 4 °C and used within a week of preparation. The filtered preparation of 25 mg/mL was freeze died to determine the concentration of Essiac tea extraction yield. Samples were found to contain 7.4 ± 0.26 mg/mL Essiac tea extract. Our study used four dilutions of this extract: 50% (3.7 mg/mL), 25% (1.85 mg/mL), 10% (0.74 mg/mL) and 5% (0.37 mg/mL) extract strengths for use in experiments.

Catalase, $FeSO_4$, H_2O_2, xanthine, xanthine oxidase, sodium formate, sodium dichromate ($Na_2Cr_2O_7$), Dulbecco's Modified Eagles Media (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Sigma (St. Louis, MO). 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was purchased from Aldrich (Milwaukee, WI). λ *Hin*dIII DNA fragments were purchased from Invitrogen (Carlsbad, CA). Vistra green nucleic acid stain was purchased from Amersham Biosciences (Piscataway, NJ). Phosphate-buffered saline (PBS) was purchased from Gibco BRL (Gaithersburg, MD). Chelex 100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA). The RAW 264.7 cell line was purchased from American Type Culture Collection (Rockville, MD). The spin trap, DMPO, was purified by charcoal decolorization and vacuum distillation. The DMPO solution, thus purified, did not contain any electron spin resonance (ESR) detectable impurities. The phosphate buffer (pH 7.4) was treated with Chelex 100 to remove transition metal ion contaminants.

2.2. Cell culture

RAW 264.7 mouse monocyte cells were cultured in DMEM with 10% FBS, 2mM L-glutamine and 50 mg/mL pen/strep at 37 °C in a 5% CO_2 incubator. Cells were split after confluence approximately every 3 days.

2.3. Free radical measurements

ESR spin trapping was used to detect short-lived free radical intermediates. This technique involves the addition-type reaction of a short-lived radical with a paramagnetic compound (spin trap) to form a relatively long-lived free radical product (spin adduct), which can then be studied using conventional ESR. The intensity of the signal is used to measure the amount of short-lived radicals trapped, and the hyperfine couplings of the spin adduct are generally characteristic of the original trapped

radicals. Spin trapping is the method of choice for detection and identification of free radical generation due to its specificity and sensitivity (Leonard et al., 2000). All ESR measurements were conducted using a Bruker EMX spectrometer (Bruker Instruments Inc. Billerica, MA 01821, USA) and a flat cell assembly. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separation using potassium tetraperoxochromate (K_3CrO_8) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reference standards (Janzen and Blackburn, 1968). The relative radical concentration was estimated by multiplying half of the peak height by $(\Delta H_{pp})^2$, where ΔH_{pp} represents peak-to peak width. Acquisit software provided by Bruker Instruments Inc. was used for data acquisitions and analyses.

Reactants were mixed in test tubes in a final volume of 1.0 mL. The reaction mixture was then transferred to a flat cell for ESR measurement. The concentrations given in the figure legends are final concentrations. Experiments were performed at room temperature and under ambient air. Control reactions of $FeSO_4 + H_2O_2$ for hydroxyl radical generation, xanthine + xanthine oxidase for superoxide radical generation and basal cellular radical generation were used in the respective experiments.

2.4. Lipid peroxidation

Lipid peroxidation of RAW 264.7 mouse peritoneal monocytes activity was estimated by malondialdehyde (MDA) production, measured by using a colorimetric assay for lipid peroxidation (LPO-586 Oxis International Inc., Portland, OR). A typical reaction mixture contained $FeSO_4$ (0.1 mM), H_2O_2 (1 mM) and 1×10^7 cells in a total volume of 1.0 PBS (pH 7.4). Essiac tea extract was added to this mixture in concentrations

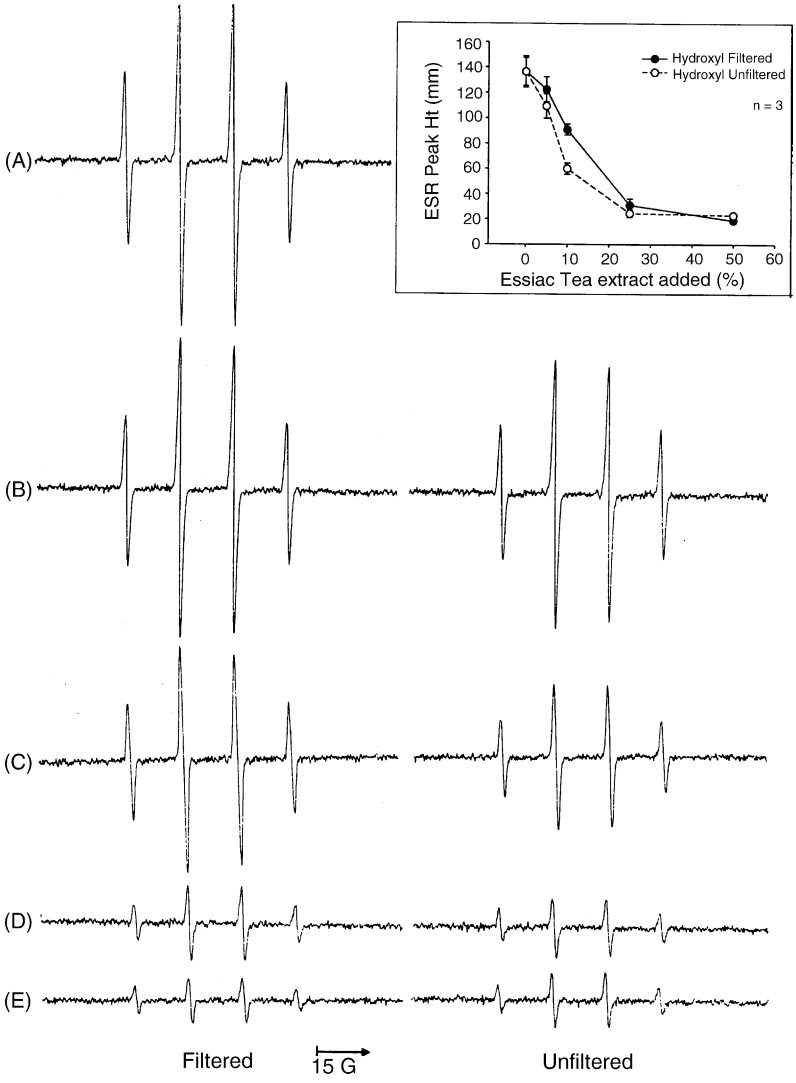

Fig. 1. ESR spectra recorded 3 min after reaction initiation in a phosphate-buffered solution (pH 7.4) containing 100 mM DMPO and the following reactants: (A) 1.0 mM $FeSO_4$ and 1.0 mM H_2O_2; (B) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2 and 5% Essiac tea; (C) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2 and 10% Essiac tea; (D) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2 and 25% Essiac tea; (E) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2 and 50% Essiac tea. The ESR spectrometer settings were: receiver gain, 2.50×10^5; time constant, 40 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3480 ± 100 G. Spectra shown are representative of three samples.

ranging from 5 to 50% to measure its effect on lipid peroxidation. The mixture was exposed for 1 h in a shaking water bath at 37 °C. The measurement of lipid peroxidation is based on the reaction of a chromogenic reagent with malonaldehyde and 4-hydroxyalkenals at 45 °C (Brambilla et al., 1989). The absorbance of the supernatant was measured at 586 nm. The percentage of inhibition caused by Essiac tea extract was calculated by comparing values to the control reaction of cellular exposure to $FeSO_4 + H_2O_2$.

2.5. DNA damage

The DNA strand break assay was carried out according to methods described earlier (Daniel et al., 1993). Briefly, reactions were performed in phosphate-buffered saline (pH 7.4) in 1.5 mL polypropylene tubes at 37 °C. A positive control reaction between $FeSO_4$ and H_2O_2 was used to generate hydroxyl radicals. Each reaction mixture contained 10 μg DNA (λ *Hin*dIII fragments), $FeSO_4$ (1 mM), H_2O_2 (5 mM) and various concentration of Essiac tea in a total volume of 100 μL of buffer. To this solution, 2 μL of gel loading buffer (50 mM EDTA, 2.5% sodium dodecyl sulfate (SDS), 0.1% bromophenol blue) was added, and then electrophoresis was performed in 0.7% agarose at 1–2 V/cm in 40 mM tris acetate buffer containing 2 mM EDTA (pH 8.0). Gels were stained in vistra green nucleic acid stain (5 μL/mL) for 30 min and photographed under UV light using a Stratagene Eagle Eye II (Stratagene Inc., La Jolla, CA 92037, USA).

2.6. Statistics

Data expressed as mean ± standard error of the mean (S.E.M.) ($n = 3$) for each group. One-way ANOVA test was performed using SigmaStat statistical software (Jandel Scientific, San Rafael, CA) to compare the responses between treatments. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Scavenging of •OH by Essiac tea extract

The Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$) was used as a source of •OH radicals. The results of scavenging •OH radicals generated with this reaction by the addition of Essiac tea extract are shown in Fig. 1. Fig. 1A shows a spectrum of an aqueous solution containing Fe^{2+}, H_2O_2 and the spin trap DMPO in a phosphate-buffered saline solution. The spectrum is a 1:2:2:1 quartet with splittings at $a_N = a_H = 14.9$ G (Janzen and Blackburn, 1968). These splitting constants and the 1:2:2:1 quartet are indicative of the DMPO/•OH adduct (Buettner, 1987; Shi and Dalal, 1989). The addition of Essiac tea extract, both filtered and unfiltered types, reduced the DMPO/•OH spectra in a concentration-dependent manner, demonstrating Essiac tea extract's ability to scavenge the •OH radical (Fig. 1B–E). Since DMPO/•OH could, in principle, arise from many sources other than ≈OH trapping we performed the competition experiment using sodium formate as a •OH radical scavenger and a source of a secondary radical to verify the presence of •OH radicals (Fig. 2). In this competition experiment, •OH radical abstracts a hydrogen atom from formate to form a new radical, which was trapped by DMPO to generate a new spin adduct signal. As expected, addition of formate decreased the intensity of DMPO/•OH adduct signal and resulted in the appearance of a new spin adduct signal with a hyperfine splittings of $a_H = 15.8$ G and $a_N = 18.8$ G (Fig. 2C). These splittings are typical of those of DMPO/≈COO^- adduct (Buettner, 1987), demonstrating that the ≈OH radicals are indeed generated. Deferoxamine was also

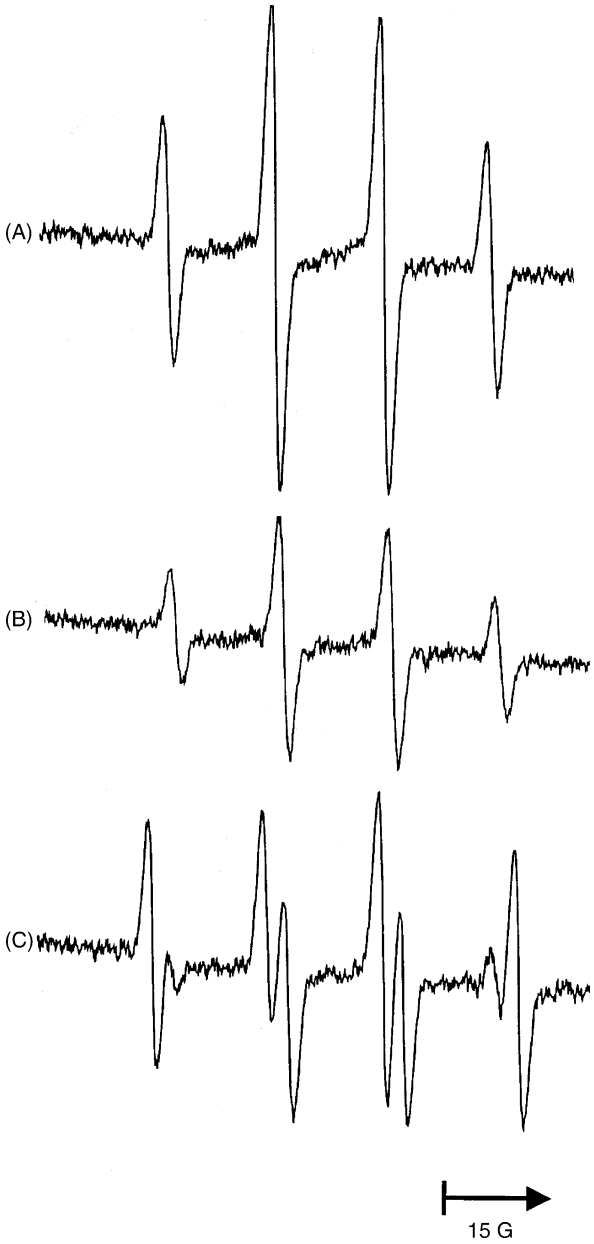

Fig. 2. ESR spectra recorded 3 min after reaction initiation in a phosphate-buffered solution (pH 7.4) containing 100 mM DMPO and the following reactants: (A) 1.0 mM $FeSO_4$ and 1.0 mM H_2O_2; (B) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2 and 50% Essiac tea; (C) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2, 50% Essiac tea and 500 mM sodium formate. The ESR spectrometer settings were: receiver gain, 2.50×10^5; time constant, 40 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3480 ± 100 G.

used to demonstrate that the Essiac was not acting as a chelator, but as a scavenger (data not shown).

3.2. Scavenging of $O_2^{\bullet -}$ by Essiac tea

Superoxide radicals were generated using a xanthine/xanthine oxidase system and measured using ESR. The spectrum in Fig. 3A shows the spin adduct spectrum generated from xanthine and xanthine oxidase in the presence of DMPO. Analysis of the spectrum shows hyperfine splittings at $a_N = 14.2$ G, $a_H = 11.5$ G, and $a_H^{\lambda} = 1.2$ G. These splittings are typical of the DMPO/$O_2^{\bullet -}$ spectra (Shi et al., 2000). Fig. 3B–E shows the reduction in signal effects of Essiac tea extract on the DMPO/$O_2^{\bullet -}$ adduct signal in a concentration-dependent manner, using both filtered and unfiltered samples.

3.3. Effect on radicals induced by cellular exposure to Cr(VI)

The generation of $^{\bullet}$OH radical from Cr(VI) stimulated RAW 264.7 cells in the presence of DMPO and the effects of Essiac are displayed in Fig. 4. The spectra in Fig. 4A and B show the spectra recorded from the spin trap DMPO and RAW 264.7 cells, respectively, in the presence of DMPO. The spectrum in Fig. 4C shows the result of RAW 264.7 cells exposed to Cr(VI) in the presence of the spin trap DMPO. The spectrum exhibits the 1:2:2:1 quartet and hyperfine splitting associated with the DMPO/$^{\bullet}$OH adduct. Fig. 4D–G show the effect of adding increasing concentrations of Essiac tea on the quantity of radicals generated by Cr(VI)-stimulated cells. A reduction in the amount of radicals trapped was observed, indicating that the $^{\bullet}$OH radicals produced by these cells are scavenged by the Essiac tea.

3.4. Inhibition of $^{\bullet}$OH-induced lipid peroxidation

Lipid peroxidation is an indicator of possible free radical damage to cells. It has been demonstrated that $^{\bullet}$OH radicals are able to cause cell membrane damage and initiate lipid peroxidation (Shi et al., 1989; Leonard et al., 2000). Fig. 5 displays the results of the measurement of lipid peroxidation by radicals generated in the Fenton reaction and the subsequent protective effects of Essiac tea in RAW 264.7 cells. In the present study, measurements were made on lipid peroxidation in untreated control cells, cells exposed to the Fenton reaction, and cells exposed to the Fenton reaction with Essiac tea treatment at various concentrations. The Essiac tea protected the cells from the radicals generated by the Fenton reaction in a concentration-dependent manner.

3.5. Inhibition of hydroxyl radical ($^{\bullet}$OH)-induced DNA damage

The Fenton reaction was used again to generate $^{\bullet}$OH radicals. Essiac tea extract was observed to protect the DNA from the $^{\bullet}$OH

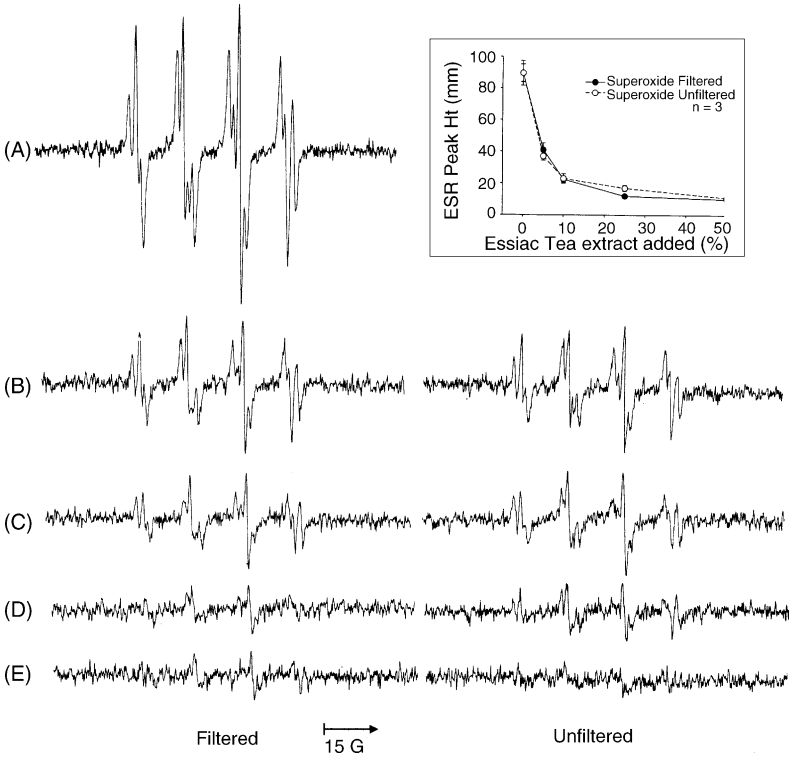

Fig. 3. ESR spectra recorded 1 min after reaction initiation from a phosphate-buffered solution (pH 7.4) containing 100 mM DMPO and the following reactants: (A) 3.5 mM xanthine and 2 U/mL xanthine oxidase; (B) 3.5 mM xanthine, 2 U/mL xanthine oxidase and 5% Essiac tea; (C) 3.5 mM xanthine, 2 U/mL xanthine oxidase and 10% Essiac tea; (D) 3.5 mM xanthine, 2 U/mL xanthine oxidase and 25% Essiac tea; (E) 3.5 mM xanthine, 2 U/mL xanthine oxidase and 50% Essiac tea. The ESR spectrometer settings were: receiver gain, 2.50×10^4; time constant, 20 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3480 ± 100 G. Spectra shown are representative of three samples.

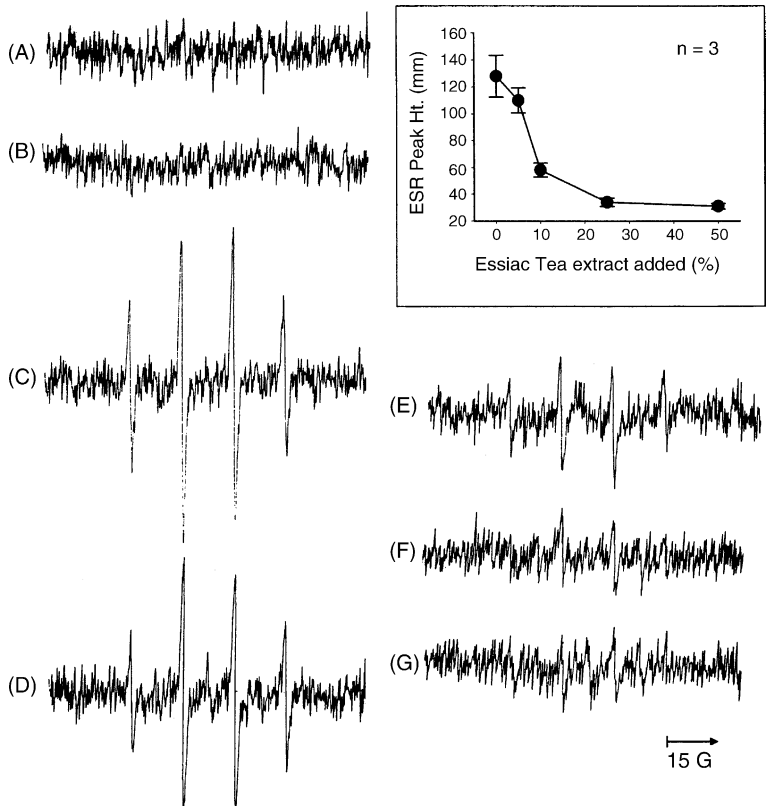

Fig. 4. ESR spectra recorded 5 min after addition of Cr(VI) to cell suspension in a phosphate-buffered solution (pH 7.4) containing the following reactants: (A) 100 mM DMPO alone; (B) 100 mM DMPO and 1×10^6 RAW 264.7 cells; (C) 100 mM DMPO, 2 mM Cr(VI) and 1×10^6 RAW 264.7 cells; (D) 100 mM DMPO, 2 mM Cr(VI), 1×10^6 RAW 264.7 cells and 5% Essiac tea (filtered); (E) 100 mM DMPO, 2 mM Cr(VI), 1×10^6 RAW 264.7 cells and 10% Essiac tea (filtered); (F) 100 mM DMPO, 2 mM Cr(VI), 1×10^6 RAW 264.7 cells and 25% Essiac tea (filtered); (G) 100 mM DMPO, 2 mM Cr(VI), 1×10^6 RAW 264.7 cells and 50% Essiac tea (filtered). The ESR spectrometer settings were: receiver gain, 5.02×10^4; time constant, 4 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3486 ± 100 G. Spectra shown are representative of three samples.

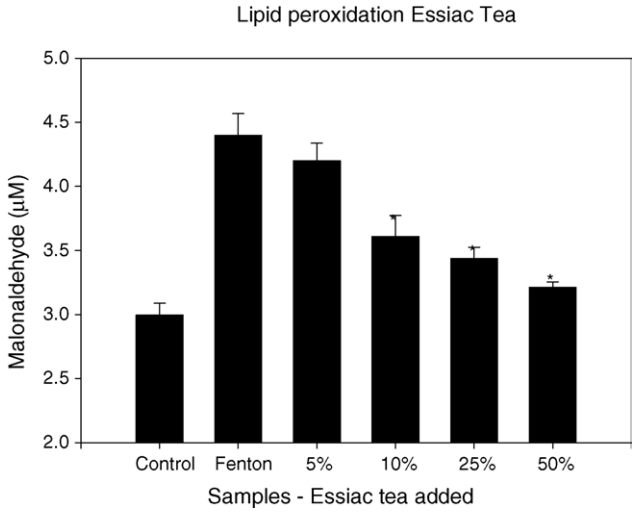

Fig. 5. Fenton reaction-induced lipid peroxidation. Exposure mixture contained 1.0 mM $FeSO_4$ and 0.1 mM H_2O_2, 5×10^7 RAW 264.7 cells and between 5 and 50% concentrations of Essiac tea (filtered). Data presented are means of ±S.E.M. for five experiments. Asterisks indicate a significant decrease in lipid peroxidation compared to the positive control Fenton reaction ($P < 0.05$).

radical "fragmenting" effect on the λ *Hin*dIII DNA (Fig. 6). Lane 1 represents untreated λ *Hin*dIII DNA alone as a control. Lane 2 shows that DNA was damaged by the Fenton reaction, resulting in smearing of the seven DNA bands. Lanes 3–6 are identical to lane 2 but with various concentrations of Essiac tea extract added. It should be noted that the smearing (lanes 3–6) was less noticeable and that the DNA bands were more pronounced than in lane 2, indicating that the Essiac tea extract was able to scavenge the $^{\bullet}OH$ radicals and protect the DNA fragments from damage.

4. Discussion

The results from the present study demonstrate that Essiac tea effectively scavenges several types of radicals and that it possesses DNA-protective effects. In non-cellular systems, Essiac effectively scavenged $^{\bullet}OH$ and $O_2^{\bullet -}$ radicals and prevented $^{\bullet}OH$-induced DNA damage. In an RAW 264.7 cell system, Essiac also scavenged induced radicals as well as effectively inhibiting lipid peroxidation. Essiac also was found to inhibit DNA damage caused by $^{\bullet}OH$ radicals.

ROS and free radicals are involved in a variety of diseases and cellular response pathways (Aust et al., 1993; Stohs, 1995; Pietraforte et al., 2002). Organisms generate antioxidants, such

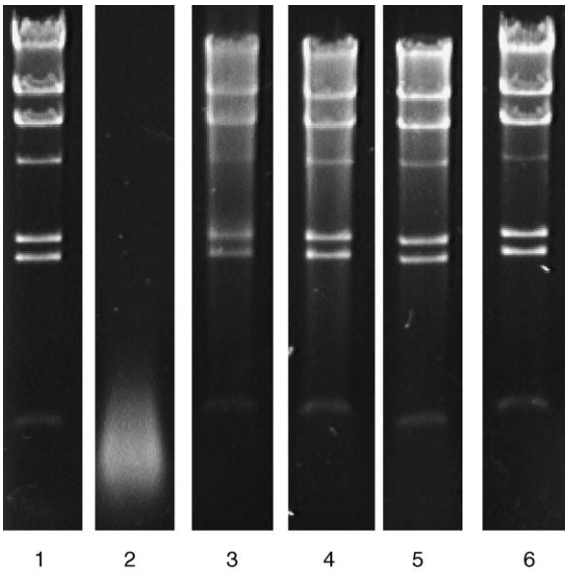

Fig. 6. DNA strand breaks in λ *Hin*dIII DNA induced by Fenton-mediated reactions. Lane 1: DNA alone; lane 2: DNA, 1 mM $FeSO_4$ and 5 mM H_2O_2; lane 3: DNA, 5% Essiac tea, 1 mM $FeSO_4$ and 5 mM H_2O_2; lane 4: DNA, 10% Essiac tea, 1 mM $FeSO_4$ and 5 mM H_2O_2; lane 5: DNA, 25% Essiac tea, 1 mM $FeSO_4$ and 5 mM H_2O_2; lane 6: DNA, 50% Essiac tea, 1 mM $FeSO_4$ and 5 mM H_2O_2.

as catalase, SOD and uric acid, which act as endogenous antioxidant defenses (Farber, 1988; Langard, 1990; Halliwell and Gutteridge, 2000). Recently, exogenous antioxidants introduced through diet or by other means have become popular. There has been a growing interest in the identification of possible dietary antioxidants to treat or prevent diseases caused by free radicals. These substances include fruits, vegetables and teas, as well as their derivatives (Wang et al., 1996; Fauconneau et al., 1997; Eberhardt et al., 2000; Karlsson et al., 2000; Leonard et al., 2002a). Epidemiologic studies have shown the effectiveness of diets rich in fruits and vegetables in reducing the risks of cancer and other diseases (Yang et al., 2000). ROS damage can be affected by two factors: (1) scavenging of radicals formed during reactions and (2) inhibiting the radical generation. The results of the present study indicate that Essiac scavenged the radicals but did not inhibit their production as measured by spin trapping competitions using sodium formate as a second free radical scavenger.

The Fenton reaction, involving Fe^{2+} and H_2O_2 for $^{\bullet}OH$ generation, was used to demonstrate Essiac's ability to scavenge $^{\bullet}OH$ radicals and establish its concentration dependence. We also used the reaction between xanthine and xanthine oxidase to demonstrate the ability of Essiac to scavenge $O_2^{\bullet -}$ radical and a Cr(VI) system to measure the effect of Essiac in a transition metal-based radical generating system.

It should be noted that Essiac was used in an aqueous medium in order to eliminate the scavenging interference presented by organic solvents, such as EtOH. This limited the concentrations which could be used to those of an aqueous environment.

The ability of Essiac to scavenge radicals generated by cellular exposure to Cr(VI) further indicates Essiac's radical scavenging ability in different types of ROS generation systems. RAW 264.7 activation by Cr(VI) has been used previously to show the oxidative stress that result from cellular stimulation by this transition metal (Shi et al., 1999). Cellular systems generate a variety of radicals including peroxisome release of H_2O_2 and electron transport chain $O_2^{\bullet -}$ generation. The scavenging seen with $^{\bullet}OH$ and $O_2^{\bullet -}$ by Essiac demonstrates its effectiveness against biologically generated radicals.

Another aspect of free radical damage involves injury to cellular membranes. Measurement of lipid peroxidation was used as an indicator of membrane damage in RAW 264.7 cells exposed to $^{\bullet}OH$ radicals generated from the Fenton reaction. Lipid peroxidation can cause a cascade effect of lipid-derived radicals, thereby causing additional membrane damage. The products of lipid peroxidation, malondialdehyde and other groups of aldehyde products, such as hexanal, 4-hydroxylnonenal and related aldehydes, may also cause DNA damage (Vaca et al., 1988). It has also been proposed that free radicals derived from lipid peroxidation may function as tumor initiators (Esterbauer, 1982). Our results showed that Essiac was effective in inhibiting lipid peroxidation of cellular membranes.

The present investigation also examined the ability of Essiac to inhibit DNA damage in λ *Hin*dIII DNA fragments from exposure to free radicals. The Fenton reaction between Fe^{2+} and H_2O_2 generates Fe^{3+}, OH^- and $^{\bullet}OH$ (Halliwell and Gutteridge, 1992). The hydroxyl radicals produced can cause DNA strand breaks. This DNA damage by free radicals has been shown to play a key role in metal-induced carcinogenesis (Paustenbach et al., 1996; Anderson et al., 2001; Chen et al., 2001; Leonard et al., 2002b; Barreto et al., 2005). The results of the present study indicate that Essiac can inhibit DNA damage caused by $^{\bullet}OH$. Carcinogens, such as chromium, asbestos, silica, nickel and possibly other metal systems, exert their carcinogenic effect, in part, through production of free radicals (Shi et al., 1989; Lison et al., 2001; Kang, 2002; De Bont, 2004; Leonard et al., 2004). The ability of Essiac to scavenge free radicals in an in vitro system demonstrates its possible preventative value in the inhibition of carcinogenesis involving free radical reactions.

In summary the results of the present investigation indicate the following: (1) Essiac is an $^{\bullet}OH$ radical scavenger; (2) Essiac scavenged the $O_2^{\bullet -}$ radical; (3) radicals produced by the RAW 264.7 cellular reaction with Cr(VI) were scavenged by Essiac; (4) lipid peroxidation in cell membranes caused by exposure to $^{\bullet}OH$ radicals was inhibited by Essiac; (5) DNA damage due to $^{\bullet}OH$ radicals produced by the Fenton reaction was inhibited by Essiac. Together, these data indicate that Essiac possesses a spectrum of antioxidant and DNA-protective properties common to anti-cancer agents.

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