Inhibition of Oxidative DNA Damage In Vitro by Extracts of Brussels Sprouts

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Accepted by Prof. B. Halliwell

(Rceived 23 November 1999; in revised form 19 January 2000)

Cruciferous vegetables have cancer preventive effects which may be due to reduction of oxidative DNA damage. We investigated the effect of an aqueous extract of cooled Brussels sprouts on formation of 7-hydroxy-8-oxo-2-deoxyguanosine (8-oxoG) in calf thymus DNA in vitro. Damage was induced by a Fenton Reaction, UVC (254 nm), DVA (365 nm), sulfolight, and methylene blue with visible light. The extract inhibited 8-oxoG formation in all systems except visible light with methylene blue. The IC₅₀ values were 6–20 μg/mL corresponding to the extract of 3–20 g of Brussels sprouts distributed in a volume of 501. The protective effect in the Fenton reaction was unaffected by addition of EDTA. After HPLC separation, fractions were identified with similar DNA protective effects. Sinigrin, a glucosinolate abundant in Brussels sprouts, co-eluted with the most effective fraction and had DNA protective effects. In comparison with other antioxidants the patterns of effect of the extract in the five-damage systems were more similar to that of sodium ascorbate than to those of dimethylsulfoxide and vitamin C. Constituents of Brussels sprouts can protect DNA by direct scavenging, e.g. hydroxyl radical and other oxidants, without preoxidant effects at concentrations potentially achievable by modest intake of the vegetable.

Keywords: Brussels sprouts, glucosinolate, ROS scavenger, 8-oxoG, DNA damage, Fenton reaction, chemoprevention, antioxidant

INTRODUCTION

Cruciferous vegetables, e.g. broccoli, cauliflower, kale, and Brussels sprouts, have significant cancer preventive effects, as shown in epidemiological and animal carcinogenesis studies. A specific cancer preventive mechanism of these vegetables could be related to a reduction of oxidative DNA damage, in particular guanine oxidation, which is the most abundant and mutagenic oxidative...
Oxidative DNA damage is frequently determined in terms of the level of the oxidatively modified nucleoside 8-oxodG, which has been widely used as a biomarker of oxidative DNA damage. Indeed, in humans a diet rich in Brussels sprouts reduced the urinary excretion of 8-oxodG. Homogenates and an aqueous extract of this vegetable had similar effects in rats, and also reduced the target organ levels of 8-oxodG after administration of the liver carcinogen, 2-nitropropane.

The apparent protective effects of cruciferous vegetables against cancer are thought to be partly due to their high content of glucosinolates, which distinguish them from other vegetables. Many of the breakdown products of these glucosinolates have potent effects on various phases I and II enzymes which could relate to the chemopreventive effects. However, the constituents of these vegetables could also include significant amounts of antioxidants with direct scavenging effects. Such DNA protective effects have previously been found in vitro and in vivo by other plant products, in particular tea and extracts thereof.

In vitro oxidative DNA damage can be induced by various radical-generating systems. Hydroxyl radicals can be generated in a Fenton reaction system, composed of iron and hydrogen peroxide. 8-oxodG is formed at high rate in DNA by visible light and a photosensitizer, such as methylene blue, which has usually been ascribed to generation of singlet oxygen. UV light, including UVA to UVC, can also induce the formation of 8-oxodG in DNA by mechanisms which may involve singlet oxygen.

In the present study the inhibition of 8-oxodG formation in calf thymus DNA by an aqueous extract of microwave cooked Brussels sprouts was investigated in a model system involving Fenton reactions. UVA, UVC, singlum light and visible light with methylene blue. In addition, the extract was separated into distinct fractions by preparative HPLC. These fractions and sinigrin, an abundant glucosinolate in Brussels sprouts, as well as some known ROS scavengers, i.e. DMSO, vitamin C and sodium ascorbate (Na2AsC), were also tested for their abilities to protect against DNA damage.

**MATERIALS AND METHODS**

**Chemicals**

Sinigrin (sulpho-glucosinolate), calf thymus DNA, 8-oxodG, 2'-deoxyguanosine (dG), sand-celite, nucleosine P1 and reagents were purchased from Sigma Co. (St. Louis, MO, USA); alkaline phosphatase was purchased from Boehringer Mannheim Co. (Indianapolis, IN, USA); ascorbic acid, DMSO, Na2AsC, and different isothiocyanates, i.e. allyl-, pheyl-, β-phenylethyl-, phenethyl-, hexyl-, propyl-, butyl-, and benzyl-ITC, were from Aldrich Co. (Milwaukee, WI, USA).

**Preparation of Brussels Sprouts Extract**

Fresh Brussels sprouts (Brassica oleracea L. var. gemmifera) were obtained from an organic grower in Oldham, DK and stored at −80°C. An aqueous extract was prepared as previously described. One kg Brussels sprouts was ground in a model MP-80 home-mixer (Bosch Multipress, Germany) with 100 ml distilled water, and the juice and residues were cooked together in a microwave oven for 3 min. The cooked materials were squeezed through gauze and the residue was repeatedly washed with distilled water until the extract was colourless. The combined solution (cooked juice and water extract from the residue) was frozen overnight at −80°C and lyophilized. The dry extract powder was kept in a closed bottle at 4°C. The powder was re-dissolved in distilled water and centrifuged at 4000 x g for 5 min before experiments. A small residue was not soluble in water or usual solvents and was discarded. An extract of green bean (Phaseolus vulgaris L.) and endive (Cichorium intybus L.) from a local market was prepared similarly.
Chromatographic Analysis of the Brussels Sprouts Extract

The aqueous solution of the extract powder of Brussels sprouts was analysed by thin-layer chromatography (TLC) and HPLC. TLC was performed on a TLC aluminium sheet with silica gel 60 F254 (Merck, Darmstadt, Germany) and developed with isopropanol-ethyl acetate-water (4:4:1). The spots on the plate were visualized under an ultraviolet lamp (254 nm) and compared with those of standard compounds, i.e. sinigrin, glucotin, allyl-ITC and phenyl-ITC.

For HPLC the extract powder solution (9 mg/ml) was decolorized on a column of sard/celite in a Pasteur pipette (glass) and then centrifuged through a Whatman filter (30 kDa, Polysulphone, Maidstone, England) at 5000 g for 30 min. The supernatant (100 μl) was loaded onto a D-7000 HPLC system (Merck, Hitachi Ltd., Japan) with a C18 Nucleosil preparative column (250 × 10 mm I.D., particle size of 5 μm, Knauer, Berlin, Germany). The column was eluted with a methanol (10%, v/v) acetate buffer (0.2 M, pH 7.0) mobile phase at a flow rate of 1.5 ml/min and fractions were collected by a model 202 fraction collector (Gilson, Middleton, WI, USA) monitored with a UV detector at 226 nm (the UV absorbance maximum of glucosinolates). The fractions corresponding to the seven major peaks of the chromatogram were pooled and lyophilized. The fractions were stored at 4°C until use.

The possible presence of catabase in the Brussels sprouts extract was assayed by incubation with hydrogen peroxide and horseradish peroxidase. The concentration of ascorbate in fresh extract powder solutions was assayed by HPLC with electrochemical detection.

DNA Exposure

The samples were composed of calf thymus DNA (300 μg) in 1.8 ml phosphate buffer (10 mM, pH 7.4) and 200 μl of test solution which consisted of a series of concentrations of either the aqueous solution of extract powder of Brussels sprouts, or sinigrin, or aliquots of each of the seven fractions obtained by preparative HPLC. Additional experiments were performed with the alternative radical scavengers, i.e. DMSO and NaN₃, and eight different isothiocyanates, which had to be dissolved in DMSO. Tween-80 or ethanol. All experiments were performed at least three times. For the Fenton system the reaction mixture included 5 or 25 μM FeCl₃ with 2 μM H₂O₂ and the reaction was performed at 37°C for 30 min by gentle shaking in the dark. In additional experiments freshly prepared EDTA (final concentration of 100 μM) was added just before iron.

For the light-induced damage the samples were placed in 12.5 ml standard plastic containers (26 cm diameter). For exposure to sunlamp light (Hohensonne 1000 lamps, without infrared bulbs, emitting light from 240 to 580 nm, Philips, Holland) a distance of 35 cm from the solution surface was used and the time was 2 min. The effect was 9.12 kJ/cm² at this exposure distance. Samples were also exposed to UVA (Hi-intensity UV lamp, B-100 filter, CA, USA) 365 nm for 30 min with an effect of 0.3 kJ/cm² or UVC (Universal UV lamp TL-900, Germany) at 254 nm for 3 min, both at an exposure distance of 6 cm. Samples with methylene blue 0.5 or 5 μM on ice were exposed to visible light from a 100 W halogen tungsten lamp for 2 min at a distance of 20 cm.

Analysis of 8-oxoG and 4G in Calf Thymus DNA

After exposure of the DNA samples 5 M NaCl was added to a final concentration of 1 M followed by 50 μl of HCl (250 mM) and two volumes (5 ml) of ice-cold 96% ethanol. The DNA was allowed to precipitate at −20°C overnight followed by centrifugation at 4000 g for 5 min. The DNA precipitate was washed with 70% ethanol, dried in air, and then dissolved in 200 μl sodium acetate buffer (20 mM, pH 4.8). The solution was digested to
nucleoside level at 37°C with 20 µl of Nuclease P1 (5 U/sample) for 30 min, and with 20 µl alkaline phosphatase (1 U/sample) for 60 min. The samples were centrifuged at 5000g for 35 min at 4°C.

An aliquot of the supernatant was injected into a chromatographic column (ODS 250 mm x 4.6 mm i.d., particle size of 5 µm, Beckman, CA, USA). The column was eluted with acetonitrile (5% v/v) in sodium phosphate buffer pH 5.7 at a flow rate of 1 ml/min. The dG in the effluent was quantified by a UV detector (Merck-Hitachi L-4000; Darmstadt) at 254 nm and the 8-oxodG by an ESA Coulochem II electrochemical detector (Bedford Inc., MA, USA) with a 9011 analytical cell set at 120 and 280 mV at electrodes 1 and 2, respectively. Quantification was performed by calibration with pure 8-oxodG and dG. The chromatographic data were processed by Merck-Hitachi software.

Statistics

I_{50} was determined by curve fitting to a log function by means of Statistic 5.0. Data were analysed with one-way analysis of variance.

RESULTS

Chromatographic Analysis and Preparative Fractionation

The aqueous extract powder of Brussels sprouts was prepared with a yield of approximately 64 g from 1 kg Brussels sprouts. Thin-layer chromatography of the aqueous extract of Brussels sprouts showed spots with R_{f} values corresponding to those of glucosinolates and isothiocyanates (Figure 1). The R_{f} values of the isothiocyanates, i.e. allyl-ITC, phenyl-ITC and others (not shown) were all very similar but distinct from those of the glucosinolates i.e. sinigrin and glucotropin (Figure 1).

The preparative HPLC of the aqueous extract consistently resulted in a chromatogram with seven major peaks when monitored at 226 nm (Figure 2). The corresponding fractions were collected for inhibition experiments and their distinct retention times were confirmed by repeating the HPLC analysis with injection of the individual peak. Pure sinigrin eluted with a retention time identical to that of peak 3 of the chromatogram (Figure 2). Under the conditions used it was not possible to obtain chromatographic peaks after injection of allyl-ITC, phenyl-ITC and other ITC compounds.

The Brussels sprouts extract contained no detectable amounts of catalase activity or ascorbate. The UV absorbance spectra and extinctions (per µg) were similar in extracts of Brussels sprouts and beans and endives.

Inhibition of 8-oxoG Formation

All the experimental systems, i.e. UVA, UVC, suslamp, a Fenton reaction and methylene blue with visible light, gave rise to the generation of 8-oxoG in calf thymus DNA (Figure 3). The 8-oxoG yield was low with UVA, possibly due to a low light fluence from the lamp. The yield was
BRUSSELS SPROUTS AND DNA DAMAGE

FIGURE 2 Chromatograms of an aqueous extract of Brussels sprouts and sinigrin by high-performance liquid chromatography (HPLC) analysis.

FIGURE 3 The relationship between the concentration of a Brussels sprouts extract and the effect on formation of 8-oxoG in cell thymus DNA by a Fenton reaction (5μM or 25μM FeCl₂, with 5μM H₂O₂ (●, O)), sunlamp light (△), methylene blue (×) with visible light (□), but without exposure (●) and by UVA (365 nm) (△), UVC (254 nm) (●), and methylene blue (×) with visible light (□). Data are mean ± SD of 3–6 experiments.

also low for UVC due to a short exposure period. Prolonged exposure, however, caused extensive fragmentation of the DNA rendering isolation impossible.

The aqueous solution of extract powder of Brussels sprouts reduced the formation of 8-oxoG in a concentration dependent manner in all systems except that with methylene blue and visible light (Figure 3) with maximal inhibition of 60–100% (Table I). However, these were substantial differences in the potency. Thus, the rank order of the IC₅₀ values of the extract was UVC = sunlamp light < Fenton system < UVA (Table II). When EDTA (100 μM) was included in the Fenton systems, the extract of Brussels sprouts inhibited the formation of 8-oxoG to the same extent as in the absence of EDTA (Figure 4). Sinigrin was less potent than the extract of
TABLE I: Effect of a Brussels sprouts extract in terms of inhibition in % of 8-oxodG formation in calf thymus DNA by reactive oxygen species generating systems

<table>
<thead>
<tr>
<th>Brussels sprouts extract (μg/ml)</th>
<th>Sunlight</th>
<th>FeCl₃ 5 μM + H₂O₂ 5 μM</th>
<th>FeCl₃ 25 μM + H₂O₂ 5 μM</th>
<th>UVA 30 min</th>
<th>UVB 254 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>36 ± 18.6</td>
<td>21 ± 7.8</td>
<td>33 ± 3.7</td>
<td>39 ± 10.0</td>
<td>55 ± 6.4</td>
</tr>
<tr>
<td>6.4</td>
<td>82 ± 6.0</td>
<td>34 ± 8.4</td>
<td>41 ± 10.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>83 ± 5.1</td>
<td>28 ± 6.6</td>
<td>48 ± 2.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>32</td>
<td>91 ± 0.4</td>
<td>67 ± 0.4</td>
<td>63 ± 10.9</td>
<td>52 ± 4.0</td>
<td>64 ± 7.4</td>
</tr>
<tr>
<td>64</td>
<td>95 ± 1.8</td>
<td>63 ± 7.0</td>
<td>70 ± 11.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>160</td>
<td>—</td>
<td>—</td>
<td>93 ± 0.0</td>
<td>86 ± 17.5</td>
<td>95 ± 1.7</td>
</tr>
<tr>
<td>400</td>
<td>—</td>
<td>—</td>
<td>93 ± 0.4</td>
<td>94 ± 7.2</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are from at least three experiments with each assay performed in triplicate; — not determined.

TABLE II: IC₅₀ values for inhibition of 8-oxodG formation in calf thymus DNA by UVA, UVC, sunlight, Fenton reaction and visible light with methylene blue (μg/ml and mM)

<table>
<thead>
<tr>
<th>Exposure systems</th>
<th>Brussels sprouts extract (μg/ml)</th>
<th>Siniginin (μg/ml)</th>
<th>NaN₃ (μg/ml)</th>
<th>DMSO (μg/ml)</th>
<th>Vitamin C (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVA</td>
<td>26.0</td>
<td>n.i.</td>
<td>26.0 (0.4)</td>
<td>prom</td>
<td>n.i.</td>
</tr>
<tr>
<td>UVC</td>
<td>5.0</td>
<td>n.i.</td>
<td>26.0 (0.4)</td>
<td>prom</td>
<td>n.i.</td>
</tr>
<tr>
<td>Sunlight</td>
<td>6.0</td>
<td>n.i.</td>
<td>26.0 (0.4)</td>
<td>prom</td>
<td>n.i.</td>
</tr>
<tr>
<td>FeCl₃ 5 μM + H₂O₂ 5 μM</td>
<td>17.5</td>
<td>n.i.</td>
<td>26.0 (0.4)</td>
<td>prom</td>
<td>n.i.</td>
</tr>
<tr>
<td>FeCl₃ 25 μM + H₂O₂ 5 μM</td>
<td>14.0</td>
<td>n.i.</td>
<td>26.0 (0.4)</td>
<td>prom</td>
<td>n.i.</td>
</tr>
<tr>
<td>Methylene blue 0.5 mM</td>
<td>n.i.</td>
<td>n.i.</td>
<td>26.0 (0.4)</td>
<td>prom</td>
<td>n.i.</td>
</tr>
<tr>
<td>Methylene blue 5 mM*</td>
<td>n.i.</td>
<td>n.i.</td>
<td>26.0 (0.4)</td>
<td>prom</td>
<td>n.i.</td>
</tr>
</tbody>
</table>

— not determined; n.i. = no inhibition; prom = promotion; * = visible light.

The IC₅₀ values were calculated by curve fitting to a log function of data from three independent sets of experiments.

Different patterns were observed when some other scavengers, i.e. DMSO, Vitamin C and NaN₃ were tested (Table II). Sodium azide was an inhibitor of 8-oxodG formation in the UVA, UVC and Fenton systems. DMSO was the most potent scavenger with respect to the Fenton system, whereas it promoted the effect of UVA with a doubling of the 8-oxodG formation at 200 μM. Vitamin C had no effect against UVA and UVC and was a strong promotor in the Fenton system with a doubling of the 8-oxodG formation at 56 μM.

![Figure 4](image) The effect of a Brussels sprouts extract on formation of 8-oxodG in calf thymus DNA by a Fenton reaction (25 μM FeCl₃ with 5 μM H₂O₂) with or without EDTA (10 μM). Data are mean with SD of 3 experiments.

The seventeen fractions from the HPLC separation of the Brussels sprouts extract were compared with respect to their abilities to inhibit 8-oxodG formation induced by the Fenton reaction,
sunlamp light, UVA and UVC (Figure 5). For this experiment each fraction was added to the exposure vials at concentrations corresponding to the yield from 90 μg/ml of the extract powder. The effects of sunlamp light and Fenton reaction were significantly inhibited by all the seven fractions, although with significantly different effects ($P < 0.01$). The maximum inhibition (75%) was achieved with fraction 3 (Figure 5a). The UVA-induced 8-oxodG levels were reduced by 50–70% by fractions 1–6 ($P < 0.05$), with fraction 3 being the most effective (Figure 5b). With UVC only fraction 2, 3, 6 and 7 demonstrated significant, but less pronounced inhibitory effects (30–50%, $P < 0.05$), again with fraction 3 as the most effective (Figure 5b).

Extracts of green beans and endives prepared similarly to the Brussels sprouts extract had no effect on 8-oxodG formation in the Fenton and sunlamp systems at concentrations up to 64 μg/ml (data not shown). With the different isothiscyanates the experiments were complicated by the solvents used, i.e. DMSCO, Tween-80 and ethanol, which had potent inhibiting effects of their own. At the necessary concentrations of solvents the formation of 8-oxodG was reduced by at least 60% in the Fenton and sunlight systems. The presence of isothiscyanates did not further reduce the formation of 8-oxodG in DNA (data not shown).

**DISCUSSION**

In the present study an extract of cooked Brussels sprouts inhibited oxidation of DNA by a number of systems generating reactive oxygen species (ROS) in vitro. Although such an effect was not seen in an extract of beans and endives, test of other vegetables will be required to show that it is specific to Brussels sprouts and possibly other cruciferous vegetables. A chromatographically separated fraction appeared to be particularly effective whereas the active compounds have yet to be identified. It appears evident that a number of different constituents contribute to the protection from oxidative DNA damage, including glucosinolates.

Previous studies in humans and rats have shown that cooked Brussels sprouts can reduce...
the spontaneous rate of oxidative DNA damage as assessed by the urinary excretion of 8-oxoG, whereas non-crucciferous vegetables had no effect.\textsuperscript{10,26} An aqueous extract similar to the present also decreased the enhanced levels of 8-oxoG in urine and the target organs, liver, kidney and bone marrow, induced by treatment of rats with 2-nitropropane.\textsuperscript{17} Although isolated indolyglycosinolates and their myrosinase generated breakdown products had no effects in rats\textsuperscript{17} other glucosinolates breakdown products could have protective effects in vivo through enhancement of phase II defence enzymes, such as glutathione peroxidase, glutathione transferases and NADPH:quinone reductase. Indeed, Brussels sprouts and isothiocyanates with allyl, aryl and/or sulphanyl side chains have such effects.\textsuperscript{19,21,22} Nevertheless, Brussels sprouts have a low content of precursors to sulphaphane, a particularly potent member of the isothiocyanates and inducer of the antioxidant responsive element effects of phase II detoxification enzymes and other protectants.\textsuperscript{21,23} Moreover, unpublished data from our laboratory show only a rather modest effect of the Brussels sprouts extract on the relevant phase II enzymes in rat liver. A number of other antioxidant vegetables products, such as tea and their extracts, have been shown to protect from 8-oxoG formation in DNA both by 2-nitropropane and other carcinogens in vivo in rats and from oxygen radical generating systems in vitro.\textsuperscript{10,24} Thus, a direct scavenging effect of some constituents could be partly responsible for the protective effects in vivo as supported by the present data. A previous in vitro study has reported relatively modest antioxidant properties or even some pro-oxidant effects of Brussels sprouts extracts prepared by methanol extraction of freeze-dried and ground material that was cooked or autoclaved.\textsuperscript{25} Similarly, some isolated glucosinolates from cruciferous vegetables, sinigrin, glucobrassicin, glucosinolates, and their breakdown products had almost no antioxidant effects.\textsuperscript{25} However, none of the assays in that study included DNA oxidation. In a deoxyribose based assay an extract from autolyzed Brussels sprouts showed iron binding capacity and modest hydroxyl radical scavenging property.\textsuperscript{25} In contrast, the DNA protective effect of the present Brussels sprouts extract in the Fenton system was independent of the presence of EDTA indicating that it is related to hydroxyl radical scavenging rather than iron chelation. Thus, differences in extract preparation and assay targets may explain the apparent differences between the present data and the study by Piambale et al.\textsuperscript{25}

Brussels sprouts contain 0.4% (w/w) glucosinolates,\textsuperscript{24} mainly sinigrin, glucobrassicin, glucoraphanin and progoitrin, of which the first two are predominant.\textsuperscript{25} In agreement with this the TLC analysis of the present Brussels sprouts extract showed spots corresponding to sinigrin, glucoraphanin and their breakdown products, such as allyl-ITC, phenyl-ITC etc. Although, all the seven fractions from the HPLC separation of the Brussels sprouts extract reduced the levels of 8-oxoG significantly in the Fenton and sunlamp systems and most of them had effects with respect to LVA and UVC, fraction 3 was particularly effective in all systems. Unfortunately, there was not sufficient material to study detailed concentration-effect relationships. Sinigrin coeluted with fraction 3 in HPLC analysis. However, on a weight basis it was less potent and could only contribute to a minor extent to the effect of the extract in Fenton reaction and sunlamp light. Eight isolated isothiocyanates, one group of the breakdown products of glucosinolates, did not seem to have DNA protective effects, although definitive conclusions cannot be drawn because of the solubility problems in the present study. Brussels sprouts are not particularly rich in the antioxidants, kaempferol and quercetin known from e.g. broccoli.\textsuperscript{26} The lack of effect of an extract of green beans and enjives in the present study and of a similar homogenate in rats in vivo indicate that the active compounds are not abundant in all vegetables.\textsuperscript{27} Accordingly, the auto-lytic process starting with myrosinase catalysed
The formation of 8-oxodG in DNA by UV light in vitro has been suggested to be due to singlet oxygen based on the enhancement by deuterium oxide and the quenching effect of sodium azide. Methylenic blue and visible light induced 8-oxodG in DNA as in many other studies. However, there was no effect of sodium azide in the present study, which could question the involvement of singlet oxygen. Nevertheless, singlet oxygen generated by methylene blue and visible light has been shown to oxidize 8-oxodG further at a high rate, which is reduced by sodium azide. Accordingly, scavenger effects may be difficult to assess in such systems although different concentrations of methylene blue yielding highly different 8-oxodG levels gave similar results concerning the scavengers. Moreover, in the present and other studies of sodium azide was also an efficient scavenger of hydroxyl radical generated in the Fenton reaction. The Brussels sprouts extract was in general similar to sodium azide with quenching of the effect of both UV and Fenton reaction on DNA. In agreement with other studies DMSO enhanced the effect of UVAs whereas vitamin C was the only compound able to quench the effect of methylene blue and visible light and a potent prooxidant in the Fenton reaction as previously shown. Thus, the pattern of effects of the Brussels sprouts extract appears similar to that of extracts of tea, in particular black tea.

Brussels sprouts are important vegetables for consumption. The yield of extract powder from Brussels sprouts was about 64g/kg. Thus, the present IC50 values of 6-20 μg/ml correspond to the intake of 5-20g Brussels sprouts assuming complete bioavailability of the active constituents and a volume of distribution of 50L. Even a modest intake of Brussels sprouts may contribute to protection against oxidative DNA damage and thus mutagenesis and carcinogenesis.

In conclusion, the present study has shown that the active compounds have yet to be identified, even a modest intake of Brussels sprouts could suffice in DNA protection if the bioavailability is not a problem.

Acknowledgements

The study was supported by the Dalocon Foundation, Vera og Carl Johan Michaelisen's Legat, Gerda og Aage Haensch Fond and The Danish Research Council (grant no. 980114).

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