**Protective role of natural antioxidants against oxidative DNA damage in HepG2 cells – evaluation by the comet assay**

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**INTRODUCTION**

DNA damage occurs in a variety of disorders such as cancer, diabetes, neurodegenerative and heart diseases where oxidative stress and free radical formation have been implicated. Consequences of fresh and plant extracts are antioxidants such as polyphenols are associated with a dominant risk of cancer and coronary heart diseases [1,2]. Today, much is known about the chemistry and antioxidant potential (as shown in a wide chemical and sub-cellular methods) of these plant antioxidants although most of the investigations on their biological effects, cellular and organ level effects remain to be done both with parent compounds and their metabolites [3]. In particular, the protective role of the DNA of dietary and natural compounds needs to be better understood. The comet assay (single cell gel electrophoresis assay) is a simple, fast, and reliable method to determine DNA damage and possible protective effects (4).

In this study, we use the comet assay to evaluate the effect of some phenolic compounds as well as Salvia extracts on protection against iron and hypochlorite (IHP)-induced oxidative DNA damage to the human hepatoma cell line HepG2. Concentrations of the phenolic compounds or extracts that reduced the IHP-induced cell death by 50% (IC50) were determined and used to evaluate their ability to specifically prevent DNA damage.

**RESULTS AND DISCUSSION**

**Evaluation of protective potential by antioxidants**

To evaluate the protective potential of the phenolic compounds and Salvia extracts we first determined the IC50 concentrations for 50% cell death with total and 50% of EHP ammended by LDH leakage and MTT assay (data not shown). Based on these results, cell incubations with 20% of ESHP were set at 3 hours to induce 45-50% of cell death. IC50 concentrations that reduced cell death by 50% of phenolic compounds were determined in incubations with the treated (Figure 1 and Table 1). Extrapolated IC50 concentrations were used in order to evaluate the protective ability to prevent oxidative damage to DNA.

- The flavonoid quercetin was more protective than phenolic acids. Of these flavonoids, rutin was the most powerful.
- Salvia, a known antioxidant, had the lowest IC50 among the compounds tested. However, concentrations greater than 25µM had progressively lost protective antioxidant effect.
- Of the Salvia extracts, the methanolic extract SOLME had more protective activity than the water extract. Of these Salvia extracts, the methanolic extract was more effective against the IHP-induced toxicity in HepG2 cells, with a IC50 value near a half of the soluble water extract.

**Evaluation of DNA damage prevention by IC50 concentration of the antioxidants**

![Graph showing DNA damage prevention by IC50 concentration of antioxidants](image1)

**Table 1 - Concentrations of the individual phenolic compounds (A) and extracts (B) that protected DNA (IC50) the cell damage induced by IHP (100 µM) cell death, as well as the comet’s histograms and IC50.**

<table>
<thead>
<tr>
<th>Component</th>
<th>IC50 (µM)</th>
<th>Histograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>1.23 ± 0.14</td>
<td><img src="image2" alt="Histogram A" /></td>
</tr>
<tr>
<td>Hydroxybenzoic acid</td>
<td>0.14 ± 0.14</td>
<td><img src="image2" alt="Histogram B" /></td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>1.16 ± 0.10</td>
<td><img src="image2" alt="Histogram C" /></td>
</tr>
<tr>
<td>quercetin</td>
<td>0.10 ± 0.02</td>
<td><img src="image2" alt="Histogram D" /></td>
</tr>
<tr>
<td>Solme (methanol)</td>
<td>0.05 ± 0.02</td>
<td><img src="image2" alt="Histogram E" /></td>
</tr>
</tbody>
</table>

**Figure 1 - Comet assay analysis of individual phenolic compounds and extracts.**

![Comet assay analysis of individual phenolic compounds and extracts](image3)

- Salvia extract and quercetin, which prevent cell death, were without protective effects at DNA level.
- Quercetin and to a lesser extent salicylic acid protected by 70% and 15%, respectively, the oxidative-induced DNA damage at IC50 concentration.
- Quercetin was effective in protecting the oxidative damage induced by IHP in a concentration dependent manner.

![Comet assay analysis of individual phenolic compounds and extracts](image4)

**METHODS**

Salmonella typhimurium, plants collected in an experimental farm located in Angra, Portugal, were collected in April, 2000, and Salvia officinalis Miller plants were collected in an experimental farm located in Braga, Portugal, and were collected in September, 2000. The aerial parts of plants were lyophilized and kept at 0°C. The method used for extraction of 80% ethanol milk (SOMME) was extracted by maceration with 100% ethanol milk (SOMME) for 7 days, followed by 100% ethanol milk (SOMME) for 7 days, followed by 100% ethanol milk (SOMME) for 7 days. The extract was then filtered and the yield of 20% [IC50] for extract and 20% [IC50] for extract were obtained. The phenolic content of SOMME was measured by UV/visible spectrophotometry.

For determination of cell viability, LDH leakage and MTT assay were performed as described elsewhere (1). The comet assay was performed using the Comet assay kit from T. Boehringer Mannheim, Germany.

**Acknowledgements**

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**References**

4. 23-04-2007, University of Minho, Campus Tecnológico de Gualtar, Braga, Portugal.

![Graph showing comparative study of antioxidant activity](image5)

**Figure 1 - Comparative study of antioxidant activity of the phenolic compounds.**

**Table 2 - Comparative study of antioxidant activity of the phenolic compounds.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Antioxidant Activity</th>
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</thead>
<tbody>
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<td>Caffeic acid</td>
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<tr>
<td>Solme (methanol)</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

**Figure 2 - Effect of IHP on the viability of HepG2 cells for 1 hour treatment of single cell comet assay.**

**Figure 3 - Effect of IHP concentration on DNA damage induced by IHP (100 µM) cell death, as well as the comet’s histograms and IC50.**

- The extent of DNA damage produced by 1 hour incubations of increasing concentrations of IHP was determined by comet assay (Figure 2). The comet images were analyzed by a semiautomated method of visual scoring. With this method, the comet images are classified according to the intensity of fluorescence in the comet tail, utilizing a value of 0, 1, 2 or 3 (fromScores to maximal damage). In this way, the tail DNA score for 100 images can range from 0 (all comet tails maximal damaged) to 100 (all maximally damaged).

- Based on the results, the comet assay was determined to be more sensitive to DNA damage than the comet assay. The DNA damage in Salvia extracts was assessed by the comet assay.

**Figure 4 - Effect of quercetin, salvia extract and caffeic acid added cell viability in the comet assay.**

- Salvia extract and quercetin, which prevent cell death, were without protective effects at DNA level.
- Quercetin and to a lesser extent salicylic acid protected by 70% and 15%, respectively, the oxidative-induced DNA damage at IC50 concentration.

- Quercetin was effective in protecting the oxidative damage induced by IHP in a concentration dependent manner.

**Figure 5 - Concentration of the phenolic compounds (A) and extracts (B) that protected DNA (IC50) the cell damage induced by IHP (100 µM) cell death, as well as the comet’s histograms and IC50.**

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**Figure 6 - Effect of quercetin, salvia extract and caffeic acid added cell viability in the comet assay.**

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