The protective effect of *Castanea sativa* leaf extract towards DNA damage induced by a singlet oxygen generating system in HaCaT cells

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INTRODUCTION

A growing body of evidence suggests that skin damage induced by UV radiation involves the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), with the consequent oxidative and nitrosative stress, resulting in structural and functional modifications in the cutaneous tissue (1). Several reactive species have been identified in the skin after UV exposure using specific ROS quenchers, namely $O_2^{\cdot-}$ (superoxide radical), $^1O_2$ (singlet oxygen), $ROO^{\cdot}$ (peroxyl radical) and $HO^{\cdot}$ (hydroxyl radical). $^1O_2$ was also directly detected in human skin following UVA irradiation by measurement of its luminescence at 1270 nm (2). This ROS is associated with DNA damage, lipid and protein oxidation. A leaf extract from *Castanea sativa* was previously shown to exhibit in vitro scavenging activity against several reactive species that are detected in the skin after UV exposure, including $^1O_2$ (3). The current study assessed the protective effect of the *C. sativa* leaf extract towards DNA damage induced by $^1O_2$ in HaCaT human keratinocytes.

MATERIALS AND METHODS

Castanea sativa leaves were collected in Mirandela (Portugal) and dried at room temperature in the dark for three weeks. The dried leaves (2 g) were grounded (500 µm) and extracted five times under magnetic stirring (10 min, 500 rpm, 40ºC) with ethanol:water (7:3) solution (5 x 50 mL) and filtered with a glass filter funnel (G4 porosity). The extracts were gathered and the ethanol was evaporated under reduced pressure at 40ºC in a rotavapor. The final dry extract was then obtained by lyophilisation. (Oxidative DNA damage, predominantly 7,8-dihydro-8-oxo-guanine, 8-oxoGua), was induced by exposing cells to the polar photosensitizer Ro19-8022 in the presence of light. HaCaT cells were incubated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum containing different concentrations of *C. sativa* leaf extract (0, 0.5, 5 or 50 µg/mL) for 2, 6 or 24 h. After washing with phosphate-buffered saline (PBS), cells were incubated with PBS containing 1 µM Ro19-8022 (Roche, Basel, Switzerland) and immediately irradiated on ice at 35 cm distance from a 500-W halogen lamp to induce oxidative modification of DNA via 1O2. The formation of 8-oxoGua was measured using the human 8-oxoguanine DNA glycosylase 1 (hOGG1) comet assay (hOGG1 comet). DNA damage was expressed as the percentage of DNA in the comet tails. Cytotoxicity was also evaluated using the trypan blue exclusion test. After 24 h incubation in medium with different concentrations of *C. sativa* extract (0, 0.5, 5 and 50 µg/mL) cells were trypsinized, mixed with trypan blue solution (0.4%) and counted in a Neubauer chamber. In this test, viable cells are those that present membrane integrity and thus do not take up the dye. Statistical analysis was performed using the software PASW 18.0. Percentage DNA damage was tested to be different from 100 with t-test and a non-parametric test (Kruskal-Wallis test), was used for the comparison of cell viability results with control (α=0.05).

RESULTS AND DISCUSSION

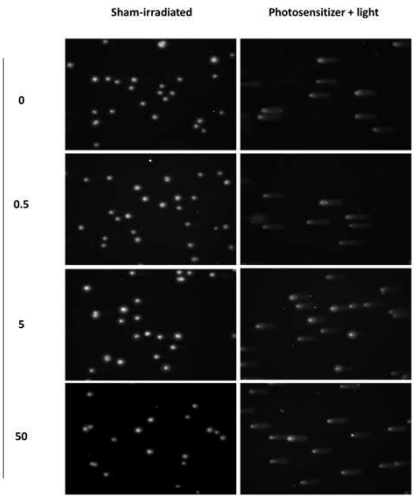

Figure 1. Effect of pre-incubation with *C. sativa* leaf extract on DNA damage induced by $^1O_2$ in HaCaT cells. HaCaTs were pre-incubated with the extract for 6 h prior to sham irradiation (left panels) or exposure to Ro19-8022 + light (right panels). Subsequently, cells were subjected to hOGG1 alkaline comet assay analysis. The percentage of the DNA in the 'tail' of the comet is proportional to the damage.

The *C. sativa* extract exhibited a concentration- and time-dependent DNA protective effect (Figs. 1, 2). Statistically significant difference from 100 % (DNA damage induced by exposing cells to the polar photosensitizer Ro19-8022 in the presence of light and without pre-incubation with *C. sativa* extract) was found after incubation with the plant extract at 5 µg/mL for 2 h (p=0.023). At 6 h a similar effect for the same concentration is depicted from figure 2 but was not statistically significant (p=0.086). Cell viability was not significantly different from control in any of the concentrations tested (p=0.981) (Fig. 3).

Figure 2. Effect of pre-incubation with *C. sativa* leaf extract on DNA damage induced by $^1O_2$ in HaCaT cells. Results are expressed as percentage of the DNA damage of control (Mean SD), n=3. Control- water:glycerin (1:1)

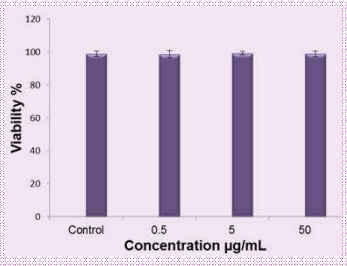

Figure 3. Viability of HaCaT cells (n=3) after incubation with *C. sativa* extract for 24 h (Mean SD. Control- water:glycerin (1:1)

CONCLUSION

In summary, results suggest that *C. sativa* leaf extract may protect the DNA from $_1O^2$– induced damage. *C. sativa* extract was also not cytotoxic to human keratinocytes after 24 h incubation in the concentrations tested in this study. These findings could be relevant in protecting the skin against the deleterious effects of UV radiation.

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Skin Forum 12th Annual Meeting
March 28th to 29th, 2011
Campus Westend, Goethe Universität Frankfurt, Germany