EFFECT OF WHITE TEA EXTRACT ON ORAL SQUAMOUS CELL CARCINOMA CELLS IN VITRO

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Abstract
Background: Polyphenols of tea have been reported to show antioxidant and antitumor effects. Highest polyphenol concentration is found in white tea, made of young leaf buds. Due to epithelial exposure in drinking tea, it is of interest to assess the effect of white tea ingredients on oral cancer cells.

Objectives: The study aimed to assess the anticancer potential of white tea extract on oral squamous cell carcinoma (SCC) cells in vitro. Materials and methods: Maceration extract was prepared by soaking white tea in ethanol and filtering, evaporation and vacuum treatment. Antioxidant activity of the extract was measured using the DPPH method. Viability of the oral SCC cell lines HSC-2 and HSC-3 was determined by tripan blue method after treatment with the extract at concentrations of 50, 100 and 400 μg/mL, using H2O2 (0.0035%) as positive control. The fraction of sub-G1 cells was measured using flow cytometry. Results and discussion: The antioxidant activity of the white tea extract was 453.3 mg AEA/100 g. Treatment of the tumor cells with 400 μg/mL of the extract significantly reduced the cell viability of both cell lines by moving more than 85% of the tumor cells to sub-G1 state. The estimated IC50 values of the extract were 259 and 365 μg/mL for HSC-2 and HSC-3 cells, respectively. Conclusions: White tea extract showed significant antioxidant activity and reduced in vitro the cell viability of the oral SCC cell lines HSC-2 and HSC-3 in a concentration-dependent manner, mainly by promoting apoptosis.

Keywords: Squamous cell carcinoma, white tea, antioxidant, tumor cell viability, apoptosis

Introduction
White tea is a special type of green tea, made from very young leaf buds still covered by the silvery white hair of the unopened leaves. White tea has a particularly high concentration of the polyphenols that have been reported to show in vitro and in vivo anticancer properties at different phases of carcinogenesis.1-3 However, there are also studies on the effects of tea or its polyphenols that have not found conclusive evidence on any anticancer benefit.

The active polyphenols of tea particularly include catechin compounds such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG), all with significant antioxidative properties.4-6 Due to repeated oral epithelial exposure to drinking tea, it is of interest to determine the effect of suggested active compounds of tea on oral cancer cells. Here this is done by exposing oral squamous cell carcinoma cells on maceration extract of white tea at different concentration levels.

Objectives
The study aimed to assess the anticancer potential of white tea extract on oral squamous cell carcinoma (SCC) cells in vitro.

Methods
Antioxidant activity of white tea was measured using the DPPH method. To check SCC cell viability and apoptosis, HSC-2 and HSC-3 cells were exposed to white tea maceration extract at concentrations of 50, 100 and 400 μg/mL. The cell viability was measured by tripan blue exclusion method. The percentage of cells at sub-G1 in the cell cycle was measured with sub-G1 apoptosis assay using flow cytometry.

Results
Antioxidant activity
The antioxidant activity of the white tea extract was 453.3 mg AEA/100 g, i.e. 100 g of the extract equals the antioxidant activity of 453.3 mg of ascorbic acid.

Viability and apoptosis of SCC cells
Significant reduction in viability of SCC cells were observed after treatment with 400 μg/mL white tea extract. The fraction of apoptotic (sub-G1) tumor cells was significantly increased after treatments with white tea extract (Table 1). Examples of the tumor cells before and after treatment with white tea extract (400 μg/mL) are shown in Figs 1 and 2.

References

Table 1. Effect of white tea extract on viability and apoptosis of HSC-2 and HSC-3 cells

<table>
<thead>
<tr>
<th>Issue</th>
<th>HSC-2</th>
<th>HSC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability untreated</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Viability 400 μg/mL</td>
<td>22.0 ± 16.5%</td>
<td>45.0 ± 19.6%</td>
</tr>
<tr>
<td>Sub-G1 untreated</td>
<td>8.0 ± 1.6%</td>
<td>12.0 ± 4.4%</td>
</tr>
<tr>
<td>Sub-G1 400 μg/mL</td>
<td>96.6 ± 0.1%</td>
<td>87.5 ± 2.0%</td>
</tr>
</tbody>
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Figure 1. HSC-2 tumor cells after treatment: untreated (left); treatment with white tea extract 400 μg/mL (right)

Figure 2. HSC-3 tumor cells after treatment: untreated (left); treatment with white tea extract 400 μg/mL (right)

Discussion
It is perhaps not surprising that conflicting results have been previously reported on the effect of white tea on tumor cells, considering the large variety in different cancers. Also, potential benefit may depend on dose, concentration or time of exposure to the active ingredients, and such variables are bound to vary in different studies. In this work, exposure to white tea extract at a concentration of 400 μg/mL significantly reduced the viability and induced apoptosis in the tested SCC cell lines. It would be of interest to study the effects also in vivo.

Conclusions
White tea extract showed high antioxidant activity. Exposure to the extract at sufficient concentration significantly reduced viability and induced apoptosis in the SCC cell lines HSC-2 and HSC-3. Further study is needed to clarify parallel benefits in vivo.

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