Antioxidant Efficacy of Hypericum Perforatum L. on 7,12-Dimethylbenzanthracene-Applied Rat Tongue Tissues

Dilara Nur Şengün¹ Gülşah Demirci² İnci Rana Karaca¹

¹ Gazi University Dentistry Faculty, Department of Oral and Maxillofacial Surgery, Ankara, Turkey
² Ankara University Medical Faculty, Department of Medical Biochemistry, Ankara, Turkey

Abstract

Background: Hypericum species have chemopreventive and antioxidant effects which have been described in the past. However, Hypericum perforatum L. extract has not been the subject of any in vivo chemoprevention studies. The purpose of this study was to investigate the effects of H. perforatum L. extract on the oxidant-antioxidant system in 7,12-dimethylbenzanthracene applied tongue tissues of rats.

Methods: Thirty male Wistar rats were divided into four groups. Control group: only paraffin, DMBA group: only 7,12-dimethylbenzanthracene, HP+DMBA group: 7,12-dimethylbenzanthracene and H. perforatum L. extract and HP group: only H. perforatum L. extract application to the oral mucosa. After a 16-week study period, animals were sacrificed and tongue samples were taken. Superoxide dismutase, catalase, glutathione peroxidase enzyme activities, and malondialdehyde and total antioxidant status levels were measured.

Results: HP+DMBA group revealed significant differences with regard to catalase and superoxide dismutase enzymes compared to control, DMBA and HP groups (Catalase: p = 0.019, 0.019, 0.000, respectively; Superoxide dismutase: p = 0.001, 0.012, 0.009, respectively). Parallel to this data, total antioxidant status value in the same group was decreased with regard to other groups. Glutathione peroxidase enzyme activity and malondialdehyde levels did not demonstrate any significant differences among groups.

Conclusions: H. perforatum L. extract did not reveal any significant evidence that indicates an antioxidant effect. Moreover, antioxidant enzymes (catalase and superoxide dismutase) were believed to be suppressed by a by-product of H. perforatum L. and 7,12-dimethylbenzanthracene reaction in the HP+DMBA group.

Key words: Antioxidants, Carcinogen, Hypericum.

INTRODUCTION

Free radicals are highly reactive and unstable molecules that have unpaired electrons. Reactive oxygen species (ROS) are by-products of the mitochondrial electron transport chain. Under normal circumstances, hazardous effects of ROS are balanced with non-enzymatic and enzymatic antioxidants in the human body. However, excessive production of ROS or any kind of defect affecting the antioxidant defense mechanisms result in oxidative stress increase in tissues and cells. It is widely known that the oxidative stress caused by free radicals and ROS plays an important role in many pathophysiological conditions including cancer (1-3).
Oral cancer is the eighth most commonly encountered cancer around the world and it may be considered a serious public health problem due to its high morbidity and mortality rates (3, 4). Recently, antioxidant agents such as natural products, herbs and medical supplements have been subject to many studies in order to discover alternative solutions to various health problems. It has been reported that plant-derived chemical compounds and products may be used as chemopreventive agents by inhibiting the initiation and progression phases of carcinogenesis. Chemopreventive agents are believed to suppress cancer formation through inhibition of ROS formation and strengthening the antioxidant defense mechanisms (4-6).

Hypericum perforatum L., also known as St. John’s Wort, has been used in different cultures as traditional medicine for decades against various health conditions. It has been reported to have wound healing, anti-inflammatory, antibacterial, antiviral, antifungal, anti-proliferative, antioxidant, antidepressant, antimalarial, antineoplastic and analgesic properties (7-13). It contains various compounds including flavonoids and proanthocyanidins, which are believed to be responsible for its antioxidant effects (8, 9).

7,12-dimethylbenzanthracene (DMBA) is a procarcinogen that exerts its effect by causing excess production of ROS and damaging the antioxidant defense mechanisms. It has been used in many animal studies in order to evaluate the chemopreventive potential of natural and synthetic products (6, 14-16).

The purpose of this study was to determine the effects of H. perforatum L. on the oxidant-antioxidant system in tongue tissues of rats that have been exposed to DMBA through oral mucosa.

MATERIALS AND METHODS

This study was approved by Gazi University Local Ethics Committee for Animal Experiments (G.Ü.ET-17.033).

Plant Material

The aerial parts of H. perforatum L. was collected in May-June 2017 during the blossoming period of plants. The plant samples were dried in room temperature and minced. The air-dried powdered plant samples were used to obtain an ethanolic extract of H. perforatum L. The crude extracts were diluted with glycerol to obtain 10 mg/ml H. perforatum L. extract. The extract solutions were stored in dark glass bottles at +4°C.

Carcinogenic material

DMBA (Sigma-Aldrich, Milwaukee, WI, USA) was used in order to induce oxidative stress in the tissues. It was prepared according to previous research protocols and diluted with liquid paraffin to 0.5% (w/v). The solution was stored in dark glass bottles at room temperature.

Animals and Experimental Design

Thirty male Wistar rats were used for the experiment. All rats were randomly divided into four groups as 6 rats in the control group and 8 rats in each of the other three groups. Liquid paraffin was applied to oral mucosa of the control group animals three days of the week (Monday, Wednesday, Friday). DMBA group received 0.5% DMBA in liquid paraffin application to the oral mucosa three times a week (Monday, Wednesday, Friday). HP+DMBA group received H. perforatum L. extract application twice a week (Tuesday, Thursday) and 0.5% DMBA in liquid paraffin application three times a week (Monday, Wednesday, Friday). HP group only received H. perforatum L. extract application twice a week (Tuesday, Thursday).

The animals were housed in propylene cages under controlled conditions of room temperature, humidity and a 12h light/dark cycle. All animals were provided with standard pellet diet and water ad libitum. All groups received 16 weeks of treatment and were sacrificed at the end of the experimental period. Tongue tissues were excised totally and stored at -80°C until used for analyses.

Biochemical analyses

All tissue samples were homogenized in physiological saline solution (20% w/v). After centrifugation of the homogenate at 5000 rpm for 30 minutes, upper clear supernatants were collected to be used in the analyses. All samples were analyzed in order to measure superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) enzyme activities and malondialdehyde (MDA) and total antioxidant status (TAS) levels.

SOD activity was measured with a method based on nitro blue tetrazolium (NBT) reduction rate. SOD activity was expressed as the amount of enzyme protein causing 50% inhibition in the reduction rate of NBT (17, 18). CAT activity was determined by measuring the absorbance of H₂O₂...
decrease at 240 nm (19). GSH-Px activity was measured by monitoring changes in NADPH absorbance at 340 nm (20). SOD activity was expressed in U/mg protein, and CAT and GSH-Px activities were expressed in IU/mg protein. MDA method is based on the absorbance measurement of thiobarbituric acid–malondialdehyde complex formation (21). TAS levels were measured with Rel Assay Total Antioxidant Status Test Kit (Mega Tıp San. ve Tic. Ltd. Şti., Gaziantep, Turkey) according to the manufacturer’s instructions. Protein amount was measured according to the Lowry method (22).

**Statistical analyses**

Data were analyzed using SPSS 11.5 software. Normally distributed values were expressed as mean ± standard deviation (SD), if not normally distributed, as median (min-max). In order to determine whether there was a statistically significant difference between the categories of a qualitative variable with three or more categories in terms of quantitative variables, one way Analysis of Variance (ANOVA) was used if normal distribution assumptions were met; if not, Kruskall-Wallis test was used. Post-hoc Tukey test was used with ANOVA in order to determine which group causes the significant difference. Bonferroni adjusted Mann-Whitney U test was used with Kruskall-Wallis variance analysis when determining which group causes the difference. The significance level was determined as p<0.05.

**RESULTS**

CAT, SOD and GSH-Px enzyme activities and MDA and TAS levels were measured in the tongue tissue samples. All measurement values are as shown in Table 1. CAT and SOD enzyme activities were found to be significantly lower in HP+DMBA group compared to the control, DMBA and HP groups (CAT: p = 0.019, 0.019, 0.000, respectively; SOD: p = 0.001, 0.012, 0.009, respectively). GSH-Px enzyme activity was also lower than all the other groups in the HP+DMBA group; however, the difference was not statistically significant. MDA levels also revealed lower values in the HP+DMBA group with respect to all the other groups, which was not statistically significant. HP+DMBA group demonstrated significantly lower TAS levels in comparison to the control group (p = 0.004).

### Table 1: CAT (IU/mg), SOD (U/mg), GSH-Px (mIU/mg) enzyme activities and MDA (nmol/mg) and TAS (µmol Trolox eq/L) levels for rat tongue tissue samples

<table>
<thead>
<tr>
<th></th>
<th>CAT Median (min-max)</th>
<th>SOD Median (min-max)</th>
<th>GSH-Px Mean±SD</th>
<th>MDA Mean±SD</th>
<th>TAS Median (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>12.17 (11.04-23.04)</td>
<td>3.76 (3.17-4.96)</td>
<td>36.83±12.91</td>
<td>1.42±0.53</td>
<td>0.27 (0.22-0.32)</td>
</tr>
<tr>
<td>DMBA (n=8)</td>
<td>12.47 (10.77-16.89)</td>
<td>3.37 (2.77-3.79)</td>
<td>32.13±2.10</td>
<td>1.18±0.15</td>
<td>0.23 (0.21-0.28)</td>
</tr>
<tr>
<td>HP+DMBA (n=8)</td>
<td>5.00 (4.05-7.14)</td>
<td>2.42 (2.14-2.65)</td>
<td>28.87±4.64</td>
<td>0.96±0.13</td>
<td>0.18 (0.15-0.24)</td>
</tr>
<tr>
<td>HP (n=8)</td>
<td>13.92 (11.51-24.09)</td>
<td>3.23 (2.85-4.93)</td>
<td>34.25±8.48</td>
<td>1.36±0.47</td>
<td>0.21 (0.19-0.37)</td>
</tr>
</tbody>
</table>

**Statistical analysis**

<table>
<thead>
<tr>
<th>Multiple comparison (p values)</th>
<th>&lt;0.001</th>
<th>&lt;0.001</th>
<th>0.272</th>
<th>0.077</th>
<th>0.006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonferroni adjusted Mann-Whitney U test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs DMBA</td>
<td>1.0</td>
<td>0.019</td>
<td></td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Control vs HP+DMBA</td>
<td>0.019</td>
<td>0.001</td>
<td></td>
<td></td>
<td>0.310</td>
</tr>
<tr>
<td>Control vs HP</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td>0.106</td>
</tr>
<tr>
<td>DMBA vs HP+DMBA</td>
<td>0.019</td>
<td>0.012</td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>DMBA vs HP</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td>0.729</td>
</tr>
<tr>
<td>HP+DMBA vs HP</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Recently, many researchers have focused on prevention of cancer, natural product and plant or herb usage in order to provide alternative management strategies against various diseases and the biological properties (antioxidant, antibacterial, antiviral, antineoplastic etc.) of various plants (3, 10, 11, 23). Chemoprevention is a novel and promising method used in experimental oncology in order to interfere with the cancer formation process using non-cytotoxic natural or synthetic agents. Chemopreventive agents are believed to function by either inhibiting the metabolic activation of carcinogens or increasing the detoxification of the metabolic residue during cancer formation, which may eventually suppress, inhibit, suspend or reverse the cancer formation process. Moreover, they may also inhibit excessive production of ROS and support the antioxidant defense mechanisms (6).

Increase in ROS production and thus oxidative stress is known to have negative effects on the endogenous antioxidants. In order to support the radical scavenging activity against oxidative stress exogenous antioxidants have been subject to many studies (24-26). Various plant-derived chemicals such as polyphenols, flavonoids and terpenes are known to have antioxidant properties (7, 27, 28). H. perforatum L. extract used in this study contains various compounds that exert in vitro antioxidant activity (29, 30).

In this study, CAT, SOD and GSH-Px enzyme activities were measured. CAT and SOD enzyme activities were significantly lower in HP+DMBA group than all the other groups. The decreased enzyme activities in the HP+DMBA group may have been caused by an interaction between the two chemicals used. The reaction between DMBA and H. perforatum L. extract may have produced a by-product that may have disrupted both enzyme activities. Same assumption may also be valid for the GSH-Px enzyme, because though insignificant, a decrease in GSH-Px activity was also observed.

Parallel to the decrease in enzyme activities, TAS level was also significantly lower in the HP+DMBA group compared to the control. This significant decrease is believed to have been triggered by the decrease in the antioxidant enzyme activities.

DMBA is a carcinogen that promotes cancer formation through ROS production (14). MDA (or TBARS) is a lipid peroxidation marker used as an indicator of oxidation or oxidative stress (31). MDA level was lower in the HP+DMBA group compared to the other groups; however, the difference was not significant.

This study did not reveal any significant data with regard to the antioxidant activity of H. perforatum L. in rat tongue tissues. Inhibition of antioxidant enzyme activities (CAT, SOD) was observed when H. perforatum L. was applied together with DMBA. Further studies are needed in order to clarify the mechanism behind the inhibition of enzyme activities and the in vivo antioxidant potential of H. perforatum L.

Acknowledgements

We acknowledge Prof. Aysel Uğur and Assoc. Prof. Nurdan Saraç for their contributions about extract preparation.

Declarations

This study was funded by Gazi University, Projects of Scientific Investigation Unit (03/2017-15). There is no conflict of interest.

References


