The anti-tumor effect of spearmint (*Mentha spicata*) in a HPV-16-transgenic mouse model

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Abstract

Infection by Human Papillomavirus (HPV) is the main cause of cervical cancer, highlighting the importance of studying compounds that may reduce viral activity and its lesions/symptoms. The aromatic herb spearmint (Mentha spicata) (MS) has proven anti-tumor properties [1]. Thus, this study aimed to evaluate the effects of an hydroethanolic extract obtained from spearmint in HPV16-transgenic (HPV+/-) mice. The extract was obtained through maceration with ethanol/water (80:20, v/v), and the phenolic composition was determined through HPLC-DAD-ESI/MS. Thirty-three female mice (16 HPV-/- and 17 HPV+/-) were randomly divided into six groups: Group (G) I – HPV-/- w/o Mentha (n=5); G II – HPV-/- w/ Mentha (0.5 mg/ml; n=6); G III – HPV-/- w/ Mentha (0.55 mg/ml; n=5); G IV – HPV+/- w/o Mentha (n=6); G V – HPV+/- w/ Mentha (0.5 mg/ml; n=6); G VI – HPV+/- w/ Mentha (0.55 mg/ml; n=5). The spearmint extract was administered in the animal's drinking water for 28 days. During the study, water and food intake as well the animals' weights were recorded weekly. Afterwards, the animals were sacrificed, and their organs were collected for oxidative stress and genetic damage analysis. A total of thirteen compounds were identified in the hydroethanolic extract, being salvianolic acid B, rosmarinic acid and luteolin-7-*O*-glucuronide, the main compounds found. Moreover, the compounds revealed to be stable in the drinking water during the 5 tested days. Results show that HPV+/- (GIV, V and VI) have lower weight but higher water and food intake, as expected. Analysis of HPV+/- mice GIV showed a significant increase of superoxide dismutase activity when compared with GV (p=0.0029) and VI (p=0.0011). This suggests that group IV was subjected to a higher oxidative stress, as expected. These results could also mean that MS is responsible for a decrease in the oxidative stress known to be induced by HPV, which in turn decreases SOD, further the role of MS as an antioxidant. Regarding genetic damage, no statistically significant changes between groups were found in the comet and micronucleus assays, implying that spearmint has no influence on genotoxicity at the concentrations employed. There were no significant differences concerning basal DNA damage between WT and transgenic individuals as seen in other studies [2]. Further studies are required to clarify the antioxidant and antigenotoxic effects of spearmint.

Introduction

HPV is responsible for inducing a series of epithelial changes, being the cause of 90% of cancers of the anus, 40% of cancers of the penis and vagina and 35% of cancers of the head and neck [3].

Mentha spicata, is an aromatic plant whose foliage is widely used in the food industry, particularly in infusions and as a condiment. Essential oils, on the other hand, are used as aromatic agents in several food products, personal hygiene, cosmetics, cleaning and pesticides [4].

K14HPV16 mice were first conceived in the 1990s, by expressing the early region of HPV-16 in basal cells of squamous epithelium of FVB / n mice, using the human cytokeratin 14 (K14) promoter, thus allowing expression to be directed for this cell type [5].

Thus, this work aims to evaluate the effect of the aromatic herb Mentha spicata as a food supplement through its incorporation in the drinking water of mice transgenic for HPV16.

Materials and Methods

The extract was obtained through maceration with ethanol/water (80:20, v/v), and the phenolic composition was determined through HPLC-DAD-ESI/MS.

Animal protocol is shown in **Figure 1**.

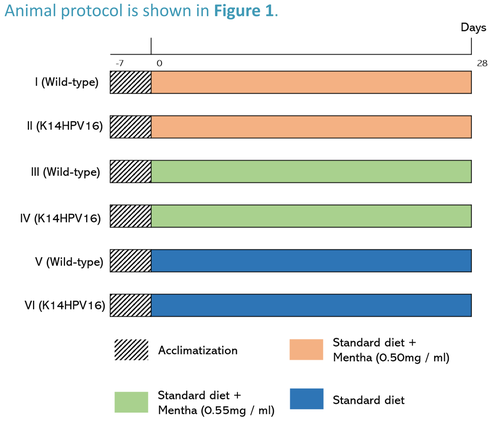

Figure 1. Experimental protocol.

Weekly, the animals were individually weighed, and water and food consumption were recorded. After 28 days, the animals were euthanized by anesthetic overdose. During euthanasia, liver samples were taken from the animals for analysis of oxidative stress and Mentha toxicity by the comet assay and micronucleus assay. All ethical issues followed the guidelines of the Portuguese *Direção Geral de Alimentação e Veterinária*.

Results

The evolution of the animals' body weight throughout the test is shown in **Figure 2**. The groups whose body weights decreased were group IV (HPV$^{(+)}$ + Mentha (0.55)) and group VI (HPV$^{(+)}$ without Mentha).

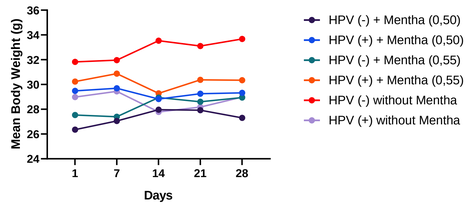

Figure 2. Mean body weight over the study days.

As we can see in **figure 3A**, the HPV$^{(+)}$ groups had a higher water consumption when compared with the wild-type groups. The group that consumed more water was the one whose Mentha was incorporated in the food at a concentration of 0.55 mg/ml. Regarding the food consumption by group, there is a higher consumption of food by the HPV$^{(+)}$ groups (**Figure 3B**).

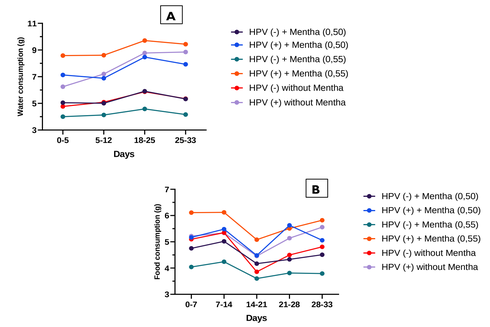

Figure 3. Mean daily water consumption **(A)** and mean daily food consumption *per* group **(B)**.

In the several tests carried out to analyze oxidative stress, only statistically significant changes were observed in the analysis of superoxide dismutase (SOD). In this, there was a significant increase in group VI (HPV$^{(+)}$ without Mentha) compared to groups II (HPV$^{(+)}$ with Mentha) (p=0.0029) and IV (HPV +/- with Mentha 0.55 mg/ml) (p=0.0011). Regarding the frequency of micronucleus, the only statistically significant change occurs between groups IV (HPV$^{(+)}$ with Mentha 0.55) and I (HPV$^{(-)}$ with Mentha 0.50) (**Figure 4A**). There were no statistically significant differences between groups regarding the comet assay (**Figure 4B**).

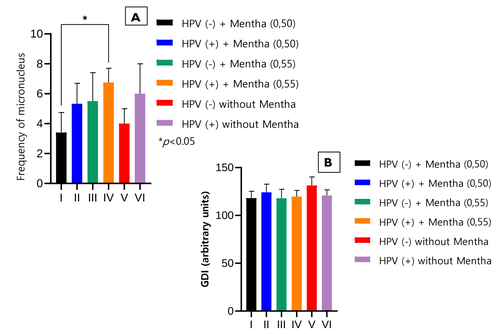

Figure 4. Mean frequency of micronuclei per 2000 erythrocytes for each group **(A)** and mean genetic damage index (GDI) by group **(B)**.

Conclusion

Based on the results obtained, it is possible to state that the animal model K14HPV16 is an excellent candidate for the study of lesions caused by HPV-16 as well as the evaluation of potential treatments. The animals did not show any side effect when exposed to the extract of Mentha spicata, nor were there any changes in biological parameters, oxidative stress or comet assay. The concentration used proved to be safe and tolerable by the animals. However, no results have been obtained that prove the antigenotoxic effect in reducing cell damage caused by HPV-16, which indicates that further studies with different concentrations and other exposure times are necessary, in order to better understand its effect and the mechanisms of action.

Refferences

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