

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

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INTRODUCTION

The Human Papillomavirus (HPV) is the prime cause of cervix cancer in women. Although there are vaccines available, there is still a constant struggle to mitigate the illness' effects. Therefore, it is important to study compounds that have the potential to slow down viral activity, reduce lesions or relieve symptoms. The aromatic herb spearmint (*Mentha spicata*) (MS) has anti-inflammatory and anti-viral properties. Thus, this study aimed to evaluate the effects of an hydroethanolic extract obtained from spearmint in HPV16-transgenic mice.

MATERIAL & METHODS

The extract was obtained from a maceration with ethanol/water (80:20, v/v), and the phenolic composition was determined through HPLC-DAD-ESI/MS. Furthermore, the stability of the phenolic compounds present in the drinking water was measured during 5 consecutive days. Thirty-three female mice (16 wild-type and 17 transgenic) were randomly divided into six groups and the MS extract was given dissolved in water for 28 day. G-I and IV receiving only tap water. Animals were sacrificed by a ketamine/xylazine overdose, blood and organs were collected. Relative organ weights were calculated by organ-to-body weight ratio and for statistical analysis a two-way ANOVA was performed, followed by the Tukey's mean comparison test ($p < 0.05$).

Groups (G)	Concentrations
G-I (HPV-/- w/o Mentha)	HPV-negative Control
G-II (HPV-/- w/ Mentha)	0.5 mg/mL
G-III (HPV-/- w/ Mentha)	0.55 mg/mL
G-IV (HPV+/- w/o Mentha)	HPV-positive Control
G-V (HPV+/- w/ Mentha)	0.5 mg/mL
G-VI (HPV+/- w/ Mentha)	0.55 mg/mL

RESULTS

A total of thirteen compounds were identified in the hydroethanolic extract, being salvianolic acid B, rosmarinic acid and luteolin-7-O-glucuronide, the main molecules present. Moreover, the compounds revealed to be stable in the drinking water during the 5 tested days. Results show that HPV-positive (G-IV, V and VI) have lower weight but higher water and food intake, as expected. Analysis of HPV-positive mice hepatic tissue has not shown any difference compared to control groups, nor there seems to be any side effect from the exposure to MS. However, G-IV demonstrated a significant increase of superoxide dismutase activity when compared to G-V ($p=0.0029$) and V-I ($p=0.0011$). This suggests that group IV is subject to a higher oxidative stress, as expected, which might help explain the higher values, as SOD is increased in these situations. 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 solidifying its role as an antioxidant. The 0.5 mg/ml and 0.55 mg/ml concentrations were also found not to be cytotoxic, showing no signs of intolerance or hepatic toxicity.

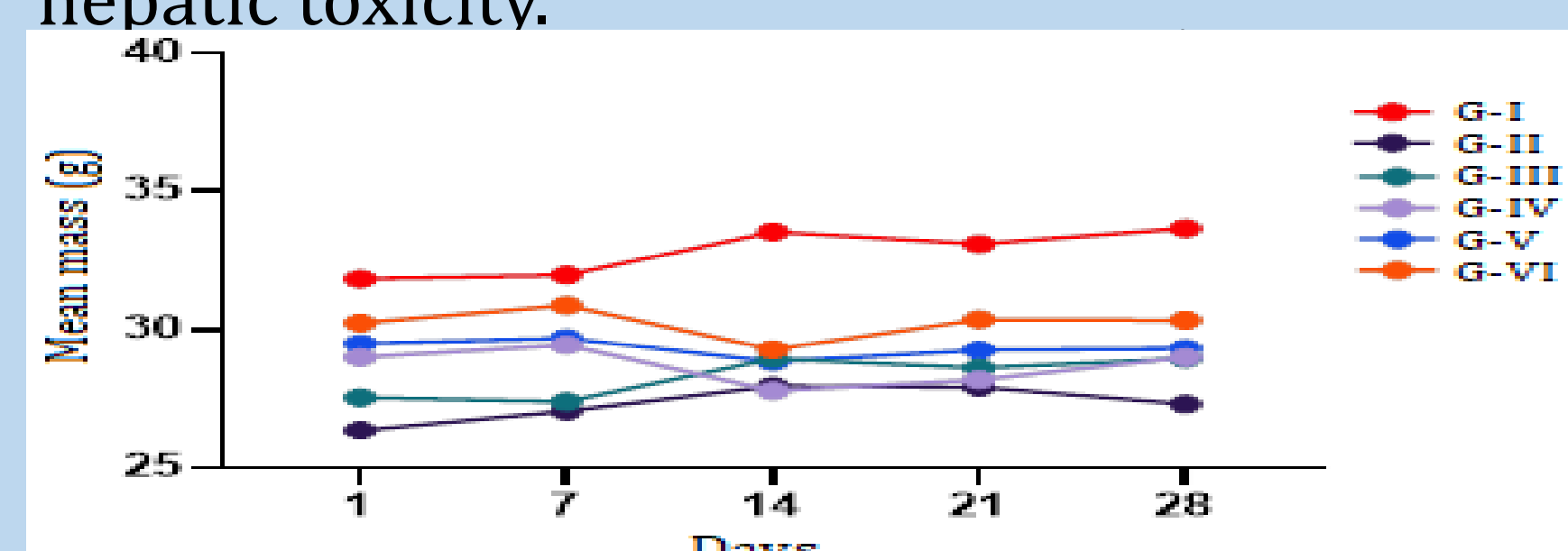


Figure 1. Means of the body weight of the groups per week during the experiment.

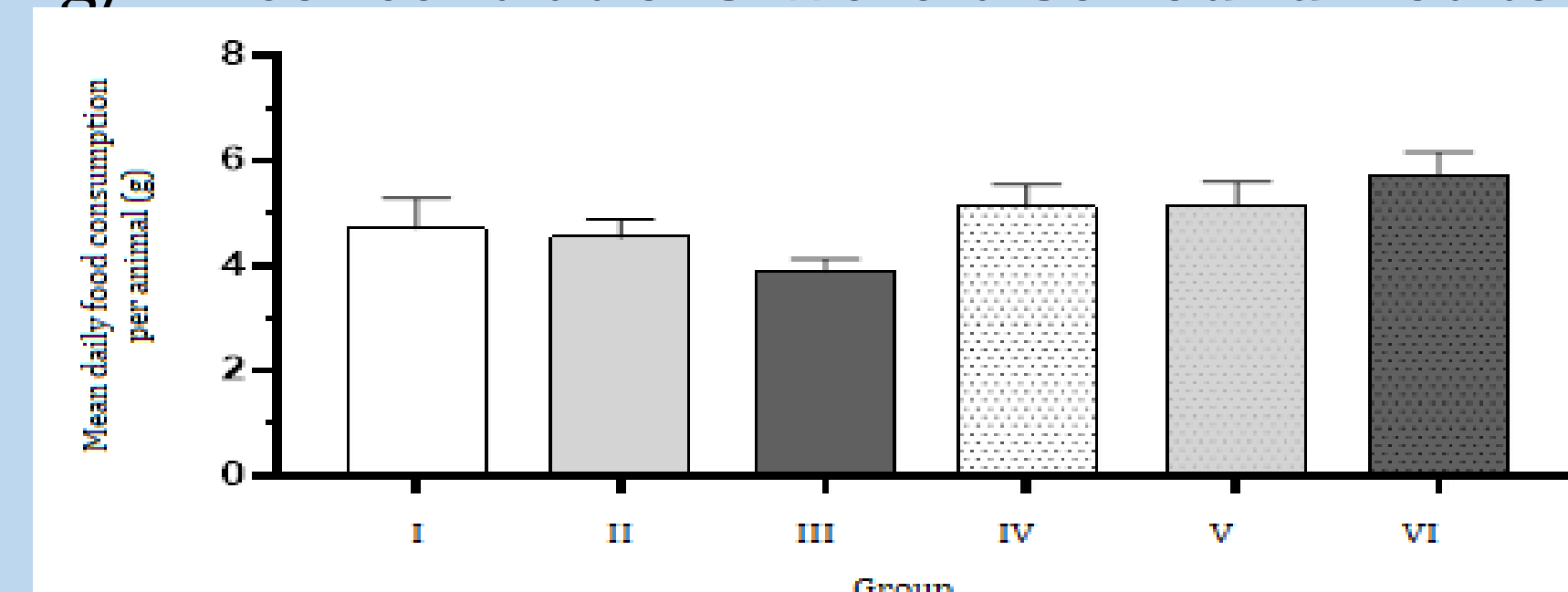


Figure 2. Mean values of food consumption per animal group during the experiment.

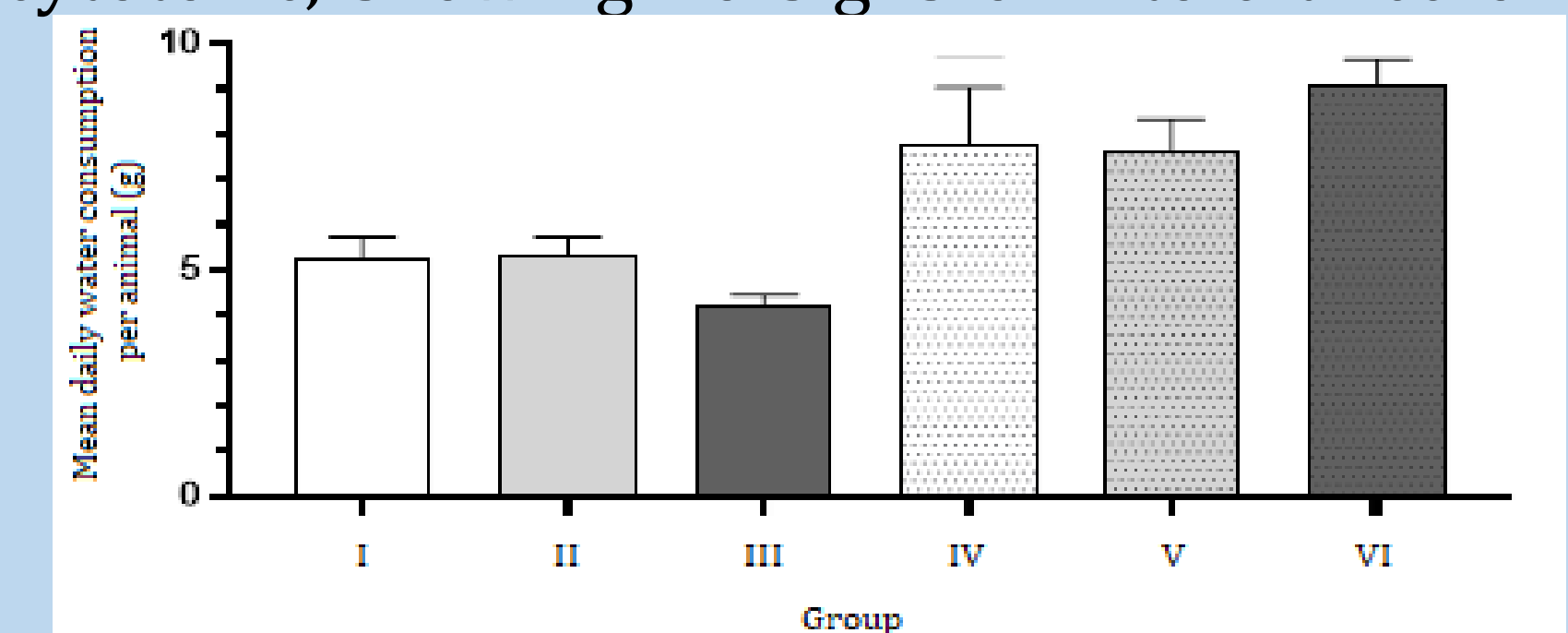


Figure 3. Mean values of drink consumption per animal during experiment.

Table 1. Relative organ weights (mean \pm standard deviation). (a) Statistically significant difference compared to groups I and III. (b) Statistically significant difference compared to groups III and VI. N

Group	Heart	Lung	Right kidney	Left kidney	Spleen	Liver
I	0,0047 \pm 0,0006	0,0065 \pm 0,0019	0,0068 \pm 0,0003	0,0068 \pm 0,0002	0,0035 \pm 0,0005	0,0478 \pm 0,0033
II	0,0048 \pm 0,0008	0,0074 \pm 0,0010	0,0070 \pm 0,0006	0,0070 \pm 0,0006	0,0046 \pm 0,0008	0,0545 \pm 0,0051 ^a
III	0,0042 \pm 0,0004	0,0066 \pm 0,0009	0,0069 \pm 0,0005	0,0069 \pm 0,0003	0,0035 \pm 0,0002	0,0496 \pm 0,0028
IV	0,0051 \pm 0,0005	0,0065 \pm 0,0011	0,0077 \pm 0,0005	0,0077 \pm 0,0004	0,0057 \pm 0,0016	0,0574 \pm 0,0069
V	0,0050 \pm 0,0006	0,0073 \pm 0,0008	0,0074 \pm 0,0006	0,0074 \pm 0,0003	0,0051 \pm 0,0012	0,0573 \pm 0,0048 ^b
VI	0,0051 \pm 0,0005	0,0059 \pm 0,0003	0,0071 \pm 0,0002	0,0071 \pm 0,0004	0,0052 \pm 0,0007	0,0529 \pm 0,0048

CONCLUSION

No statistically significant difference was recorded in the weight growth between groups. Comparing to other studies, one obtained the same effect with linden¹ and another one observed a severe weight loss with laurel². A higher water and food intake was observed on HPV-positive groups and is on par with other authors findings^{3,4}. However, on the case of HPV-negative groups, the opposite was verified – the higher the MS concentration, the lower the food consumption. Another study had similar results⁵.

Further studies are required to clarify the correlation between superoxide dismutase and spearmint, and its toxicity.

Acknowledgments

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Bibliography

- Macedo, S., Borges, I., Nogueira, A., Barros, L., Ferreira, I. C. F. R., Neuparth, M. J., ... Oliveira, P. A. (2019). Avaliação in vivo dos efeitos fisiológicos resultantes da exposição à tília. *XII Jornadas de Bioquímica*. Vila Real: UTAD.
- Medeiros-Fonseca, B., Mestre, V. F., Colaço, B., Pires, M. J., Martins, T., Gil da Costa, R. M., ... Oliveira, P. A. (2018). Laurus nobilis (laurel) aqueous leaf extract's toxicological and anti-tumor activities in HPV16-transgenic mice. *Food & Function*, 9(8), 4419–4428.
- Gil da Costa, R. M., Aragão, S., Moutinho, M., Alvarado, A., Carmo, D., Casaca, F., ... Oliveira, P. A. (2017). HPV16 induces a wasting syndrome in transgenic mice: Amelioration by dietary polyphenols via NF- κ B inhibition. *Life Sciences*, 169, 11–19.
- Nobre, R. J., Herráez-Hernández, E., Fei, J.-W., Langbein, L., Kaden, S., Gröne, H.-J., & de Villiers, E.-M. (2009). E7 Oncoprotein of Novel Human Papillomavirus Type 108 Lacking the E6 Gene Induces Dysplasia in Organotypic Keratinocyte Cultures. *Journal of Virology*, 83(7), 2907–2916.
- Barbalho, S. M., Spada, A. P. M., Oliveira, E. P. de, Paiva-Filho, M. E., Martuchi, K. A., Leite, N. C., ... Oshiiwa, M. (2009). Mentha piperita effects on wistar rats plasma lipids. *Brazilian Archives of Biology and Technology*, 52(5), 1137–1143.