

# EFFECT OF HIDROETHANOLIC EXTRACT OF *LAVANDULA PEDUNCULATA* (MILL.) CAV. ON MORPHOMETRIC PARAMETERS IN HPV-16 TRANGENIC

Nascimento-Gonçalves E., Ferreira T., Moutinho M. S. S., Pires M. J., Bastos M. M. S. M., Medeiros R., Nogueira A., Barros L., Ferreira I. C. F. R., Gil da Costa R., Oliveira P.A.\*

\*pamo@utad.pt

## Abstract

*Lavandula pedunculata* (Mill.) Cav., common English name French lavender, belongs to the Lamiaceae family and has been used as a medicinal plant in infusions for respiratory and digestive systems and as a therapeutic agent with antiseptic action for cleaning wounds [1,2]. The K14HPV16 mice is a skin squamous carcinoma model that can be used to test the antitumoral properties of several chemical and natural products [3]. The aim of this work was to evaluate the effect of the hydroethanolic French lavender extract (FLE) on body weight, relative organs weights, food and water consumption in an HPV-16-transgenic mice model. 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. Twenty-eight male mice were randomly divided into four groups (n=7/group) according to their genotype: group I (HPV16- control); II (HPV16- FLE); III (HPV16+ control) and IV (HPV16+ FLE). The FLE was administrated orally in drinking water at 6.8 mg/10mL/animal to animals from groups II and IV and changed every 4 days. The animals were kept under controlled conditions such as temperature, light and humidity. Food and water were kept *ad libitum* regime. Animals' body weight, food and water consumption were measured weekly as well as animal welfare. After twenty-nine days, all animals were sacrificed by anaesthetic overdose and blood was obtained from cardiac puncture. The organs were collected and immediately weighted. Data was analysed using SPSS 25. The differences were considered statistically significant at p<0.05. A total of thirteen compounds were identified in the hydroethanolic extract, being salvianolic acid B and rosmarinic acid the main molecules present. Moreover, the compounds revealed to be stable in the drinking water during the 5 tested days. HPV animals exposed to FLE (group IV) showed lower values of body weight variation than HPV animals not exposed to lavender (group III) in week 1, 2 and 3 (p<0.05), suggesting that the FLE was highly palatable. However, the values of food consumption were identical between groups and water intake was higher in transgenic animals as expected. The relative organ weight of heart, lung, kidneys, adrenals and liver did not demonstrate differences between groups (p<0.05). According to our results the consumption of French lavender demonstrated a favourable and safe toxicological profile using these experimental conditions.

## Introduction

The K14HPV16 mice model expresses all the early HPV16 genes under of the cytokeratin 14 (*Ck14*, *Krt14*) gene promoter and develop cervical and cutaneous lesions. Thus, the K14HPV16 mice is a skin squamous carcinoma model that can be used to test the antitumoral properties of several chemical and natural products [3].

*Lavandula pedunculata* (Mill.) Cav., common English name French lavender, belongs to the Lamiaceae family and has been used as a medicinal plant in infusions for respiratory and digestive systems and as a therapeutic agent with antiseptic action for cleaning wounds [1,2].

The aim of this work was to evaluate the effect of the hydroethanolic French lavender extract (FLE) on body weight, relative organs weights, food and water consumption in an HPV-16-transgenic mice model.

## Methodology

Study design and animal procedures were approved by the University of Trás-os-Montes and Alto Douro Ethics Committee (10/2013) and the *Direção Geral de Alimentação e Veterinária* (0421/000/000/2014). The K14HPV16 model on FVB/n background was generously were generously donated by doctors Jeffrey Arbeit and Douglas Hanahan, from the University of California, through the National Cancer Institute's Mouse Repository. The animals were kept under controlled conditions of temperature (23 ± 2°C), light-dark cycle (12 h light/12 h dark), and relative humidity (50 ± 10%). Food and water were kept *ad libitum* regime.

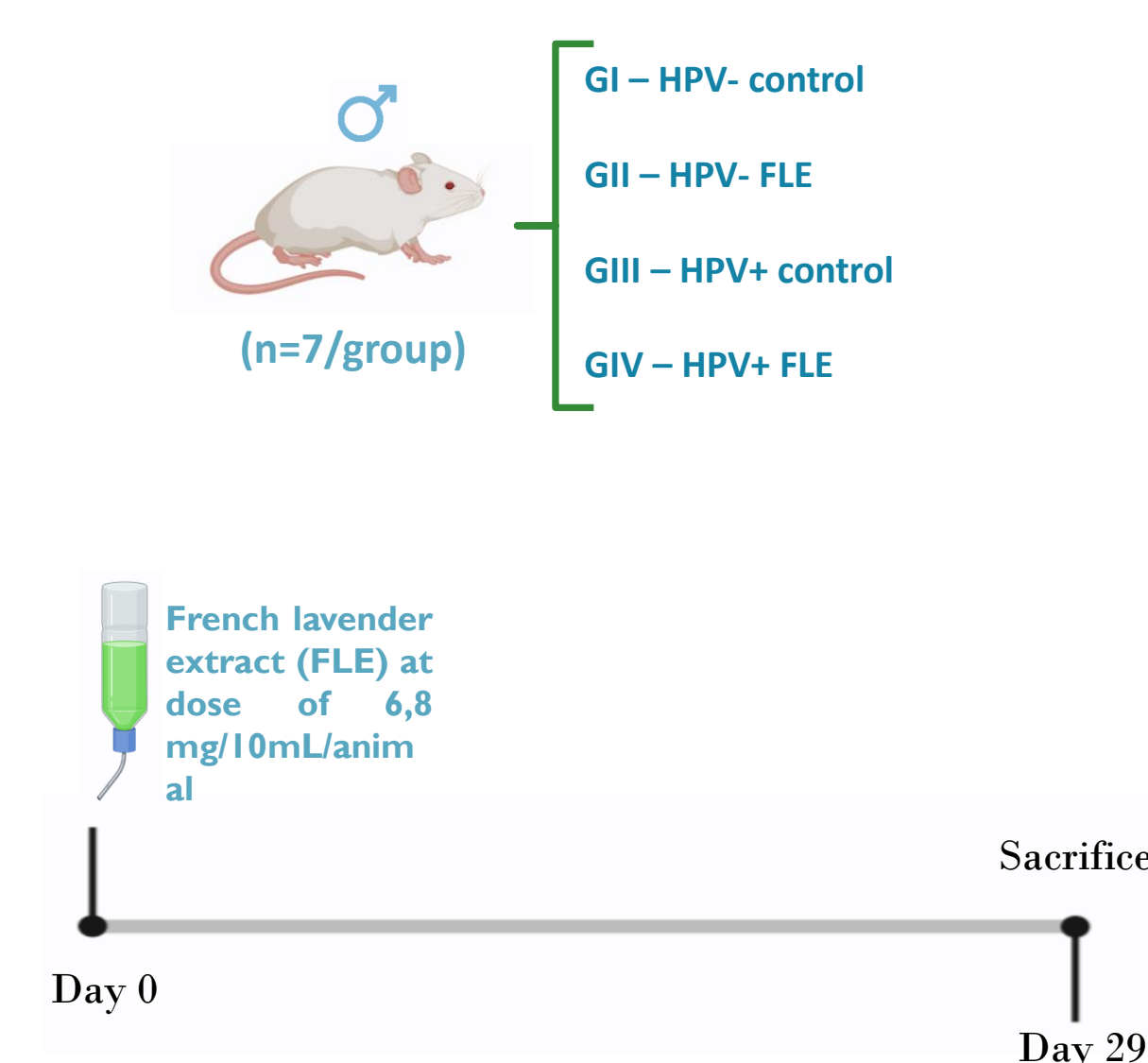


Figure 1 Experimental design

Animals' body weight, food and water consumption were measured weekly as well as animal welfare. After twenty-nine days, all animals were sacrificed by anaesthetic overdose and blood was obtained from cardiac puncture. The organs were collected and immediately weighted.

SPSS 25.0 software (Statistical Package for the Social Sciences Chicago, Illinois, USA) was used for data processing. Measurement data were presented as mean ± standard error (SE) and processed in one-way analysis of variance (ANOVA) with Bonferroni multiple comparison post-hoc analysis. The statistical significance was defined when  $p < 0.05$ .

## Results

A total of thirteen compounds were identified in the hydroethanolic extract, being salvianolic acid B and rosmarinic acid the main molecules present. Moreover, the compounds revealed to be stable in the drinking water during the 5 tested days. HPV animals exposed to FLE (GIV) showed lower values of body weight variation than HPV animals not exposed to lavender (GIII) in week 1, 2 and 3 (p<0.05), suggesting that the FLE was not highly palatable (Figure 1). Additionally, Wild-type animals exposed to FLE (GII) showed higher values of body weight than HPV animals not exposed to lavender (GIII). However, the values of food consumption were identical between groups and water intake was higher in transgenic animals as expected (data not show).

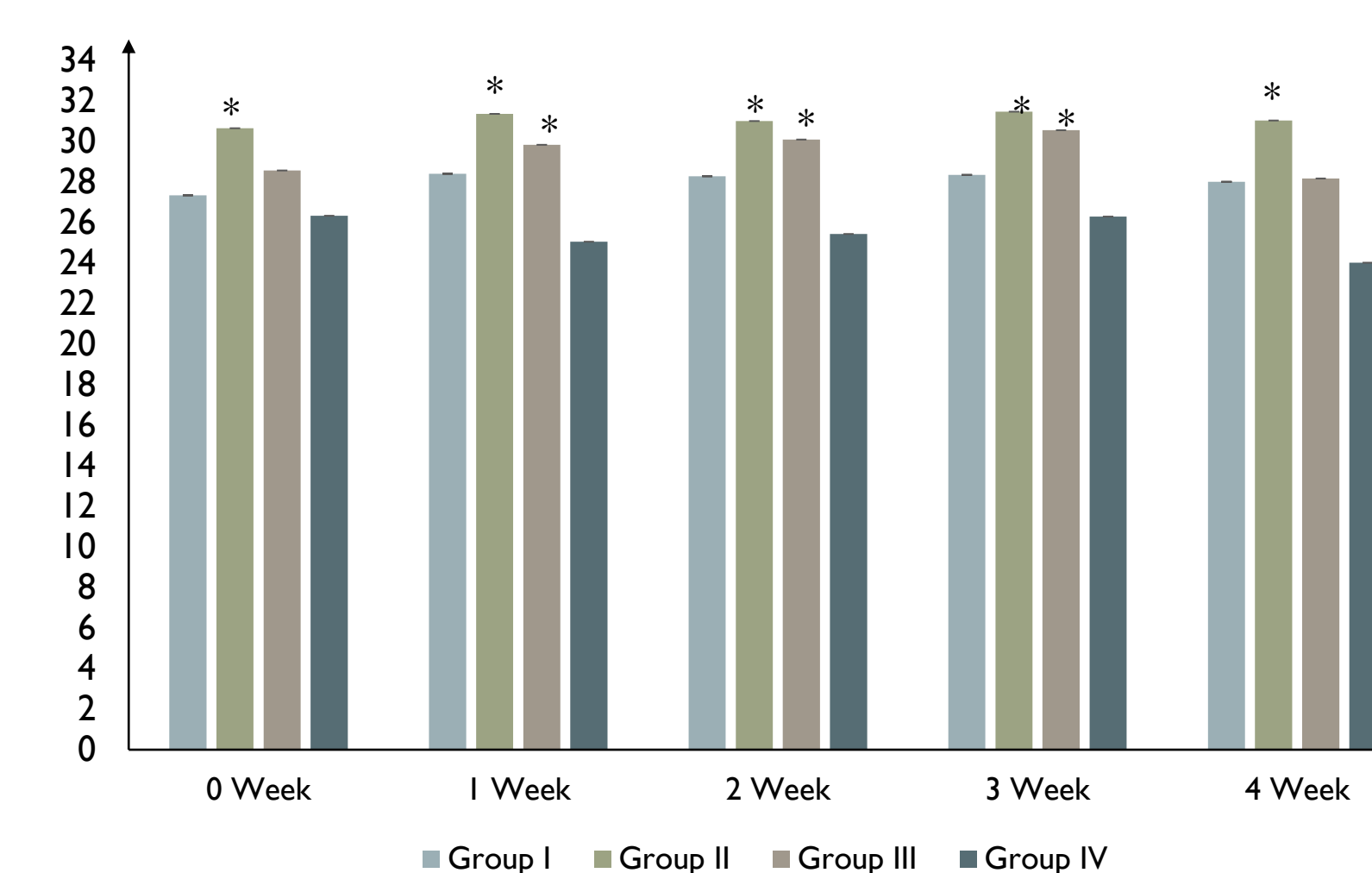


Figure 2. Weekly variation of body weight (g). \*Statistically significant difference from group IV ( $p < 0.05$ ).

The relative organ weight of heart, lung, kidneys, adrenals and liver did not demonstrate differences between groups ( $p < 0.05$ ) (table 1). Spleen relative weight of wild-type animals (GI and II) shown higher values compared to HPV control (GIII). Moreover, the thymus relative weight of GI shown higher values compared to HPV animals (GIII and IV). These results suggest that only oncogenes HPV had effect in these organs.

Table 1. Relative organ weights.

Groups	Heart	Lung	Right kidney	Left kidney	Right adrenal	Left adrenal	Spleen	Liver	Thymus
GI	0.0049 ±0.0003	0.0061 ±0.0002	0.0081 ±0.0004	0.0078 ±0.0003	0.0002 ±0.0.00007	0.00022 ±0.00006	0.0038 ±0.0002*	0.0520 ±0.0010	0.0011 ±0.0001 <sup>a</sup>
GII	0.0044 ±0.0002	0.0059 ±0.0003	0.0079 ±0.0002	0.0074 ±0.0003	0.0002 ±0.0.00003	0.0004 ±0.00006	0.0034 ±0.0002*	0.0534 ±0.0011	0.0007 ±0.0001
GIII	0.0051 ±0.0002	0.0062 ±0.0003	0.0080 ±0.0004	0.0078 ±0.0005	0.0001 ±0.0.00005	0.0002 ±0.00010	0.0074 ±0.0017	0.0539 ±0.0028	0.0005 ±0.0011
GIV	0.0049 ±0.0001	0.0065 ±0.0004	0.0083 ±0.0003	0.0076 ±0.0002	0.0001 ±0.0.00004	0.0002 ±0.00003	0.0059 ±0.0007	0.0542 ±0.0026	0.0004 ±0.0001

<sup>a</sup> Statistically significant difference from group III ( $p < 0.05$ ); <sup>b</sup> Statistically significant difference from group IV ( $p < 0.05$ ).

## Conclusion

According to our results the consumption of French lavender demonstrated a favourable and safe toxicological profile using these experimental conditions. Further studies will be needed as a histological analysis to assess the toxicity of the compound, especially in the kidney and liver. In addition, skin histology will be performed in order to evaluate the chemopreventive effect of lavender on HPV-induced lesions 16.

## References

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