Assessing the *in vivo* effects of *Aloysia citrodora* extract: data from K14-HPV16 transgenic mice

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BACKGROUND

Aloysia citrodora is used in folk medicine in the preparation of infusions due to its antispasmodic, digestive, sedative, and antipyretic properties. It also has antioxidant and antimicrobial properties.

PURPOSE OF THE EXPERIMENTAL

The present study evaluated the *in vivo* efficacy of aqueous extract (AE) of *Aloysia citrodora* in K14-HPV16 transgenic mice which HPV16 early genomic region and hence develop multiple-step epithelial neoplasia.

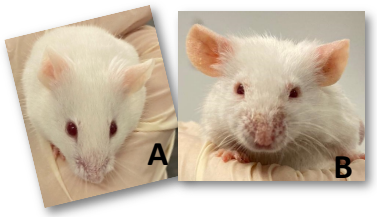

Figure 1. Appearance of wildtype **(A)** and K14-HPV16 **(B)** mice.

METHODS

Thirty wildtype (WT) or K14-HPV16 (**figure 1**) female mice were divided into six groups (G) (n=5): G1 (WT, control), G2 (HPV, control), G3 (WT, 0.013g/mL), G4 (HPV, 0.006g/mL), G5 (HPV, 0.008g/mL), G6 (HPV, 0.013g/mL). AE was prepared and provided in drinking water every 48 h; the experiment lasted 28 days. Body weight, food and water consumption and humane endpoints were recorded weekly. At necropsy, chest and ear skin was collected and processed for histological analysis (**figure 2**).

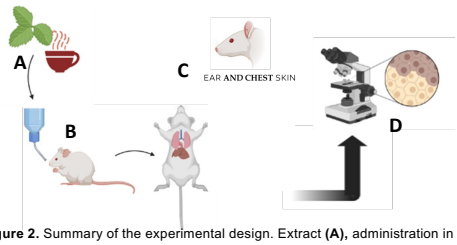

Figure 2. Summary of the experimental design. Extract **(A),** administration in drinking water **(B)**, samples collection **(C),** and histological analysis (D).

RESULTS

The mean body weight of all animals increased throughout the experiment (p<0.05). Food and water intake were slightly higher in the HPV groups. The humane endpoints score was different between G2 and G4, and G4 and G5 (p<0.05) (**table 1**). The skin lesions in K14-HPV16 mice comprised hyperplasia, dysplasia, benign tumours (papillomas) and malignant tumours (carcinomas), with the worst lesions registered on the ear pavilion in G2, followed by G5 (p<0.05). The highest dose group (G6) showed a lower number of malignant tumours (p<0.05) (**figure 3-5**).

Table 1. *Humane endpoints* of different groups of animals (M ± SD) during the experimental trial.

G1 WT water	0.08 ± 0.18
G2 HPV water	0.36 ± 0.41[1]
G3 WT 0.013 g/mL	0.24 ± 0.22
G4 HPV 0.006 g/mL	0.36 ± 0.26
G5 HPV 0.008 g/mL	1.04 ± 0.09[2]
G6 HPV 0.013 g/mL	0.80 ± 0.40

(1) Statistically different from group 4 (p < 0.05). (2) Statistically different from group 4 (p < 0.05).

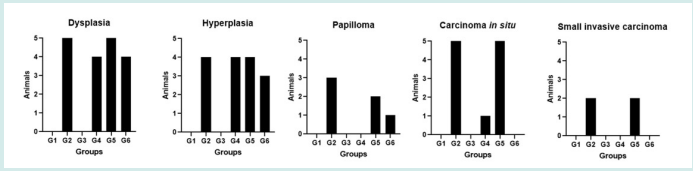

Figure 3. Histological results of ear skin.

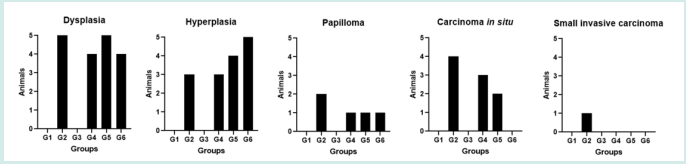

Figure 4. Histological results of chest skin.

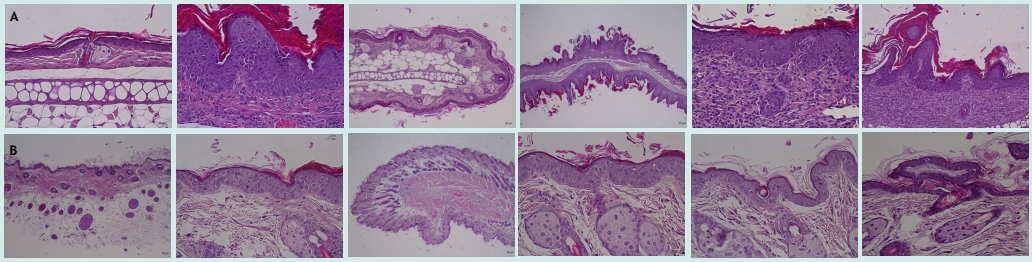

Figure 5. Representative images of the skin lesions observed in rats under study (H&E staining). Ear skin (A) and chest skin (B) in G1, showing no significant histopathological changes; carcinoma invasive (B) in ear skin G2, (G3) normal, (G4) papillomatosis, (G5) carcinoma *in situ*, (G6) papilloma. Chest skin, in (G1) normal, (G2) carcinoma *in situ*, (G3) normal, (G4) dysplasia, (G5) hyperplasia, (G6) papilloma.

CONCLUSION

The results suggest that AE is safe for the studied animals, showing a dose-dependent trend towards lesion improvement. As AE is a natural product, the authors believe that further studies including higher doses are now warranted.

ACKNOWLEDGMENTS

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