Histopathological evaluation of mice's liver and kidney after exposure to an elderberry extract

T. Azevedo [1*], T. Ferreira [1], L. Barros [2], I.C.R.F. Ferreira [2], M.L. Pinto [3], P.A. Oliveira [1]

[1] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; [3] Animal and Veterinary Research Centre (CECAV), UTAD, Vila Real, Portugal;

* tiagoaazevedo99@gmail.com

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Introduction

Elderberry (*Sambucus nigra* L.) is recognised for its use as a **food source**, **food additive**, in **nutraceuticals**, and in **folk medicine**. Its berries are highly concentrated in **flavonoids**, particularly **anthocyanins**, well-known for their colorant and antioxidant properties. The main **goal** of this study was to evaluate the **influence** of an **anthocyanin-rich elderberry extract** (EE) on **mice** for 29 days and to assess its safety when used as **natural food colorant**.

Methodology

The anthocyanin profile was determined using **HPLC-DAD-ESI/MS**. This study (Fig. 1) was approved by the University of Trás-os-Montes and Alto Douro Ethics committee (approval no. 10/2013) and the Portuguese Veterinary Authorities (approval no. 0421/000/000/2014). The animals were kept under controlled conditions. Food and drink were kept *ad libitum* regime.

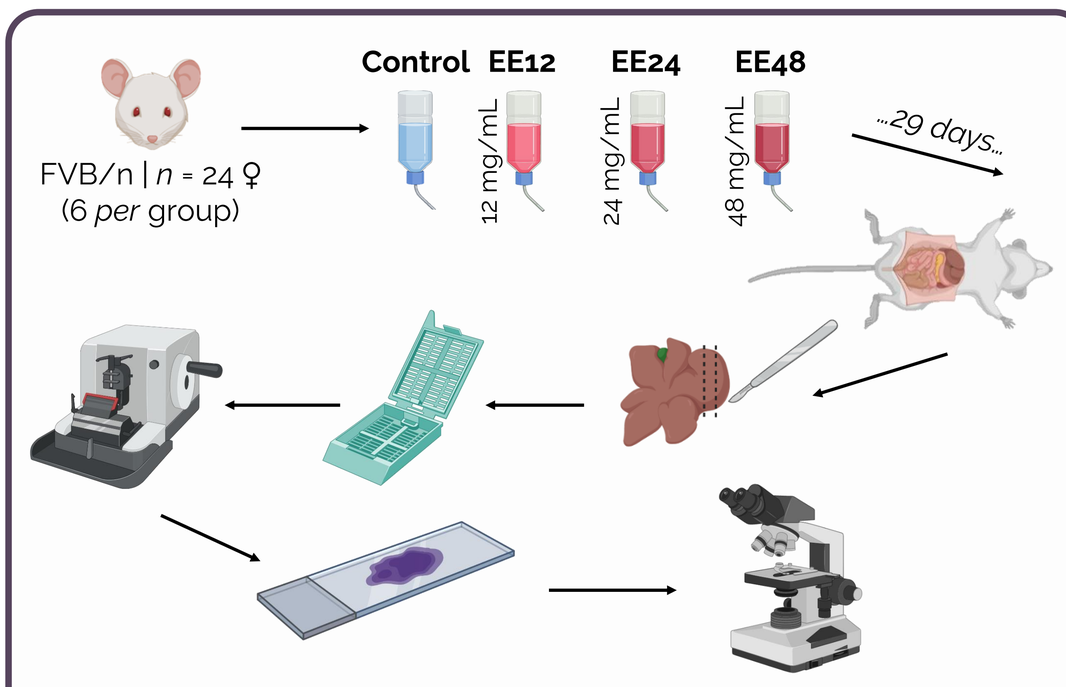

Fig. 1 | Experimental design from the animal protocol to the hepatic and renal histology analysis.

EE was dissolved in normal water in three increasing concentrations (EE12, 24 and 48) and changed every **2-3 days** due to the compounds' stability. The control group was supplemented only normal water. The same researcher assessed **Humane endpoints** on a **weekly basis**. Animals were humanely sacrificed by intraperitoneal injection of **xylazine and ketamine**, followed by **exsanguination** by cardiac puncture. A **complete necropsy** was performed. For **histopathological analysis**, **liver** and **kidney** samples were collected and **fixed in 10% buffered formalin**, sectioned, and processed for paraffin embedding. Sections were **stained with haematoxylin and eosin** and observed under **light microscope**.

Results

The predominant anthocyanins detected were cyanidin-3-*O*-sambubioside-5-*O*-glucoside, cyanidin-3-*O*-sambubioside and cyanidin-3-*O*-glucoside. No animals die during the experimental work. In addition, the evaluation of Humane endpoints revealed **no behavioural changes** in the animals after EE supplementation. Histological analysis of the liver (Fig. 2) showed a **significant difference** (p = 0.036) between Control (80.0%) and EE12 (16.7%), with the **control groups' histology exhibiting overall hydropic changes**. These changes presented a **generalized distribution** in Control mice, whereas the other groups exhibited hydropic changes at the **centrilobular zone**. **Inflammation** in the **liver** was also assessed, with **at least one animal** in each group exhibiting chronic focal hepatitis. Apart from **one** case of **chronic interstitial nephritis** in EE24, **no histological changes** were observed regarding the kidney's histology.

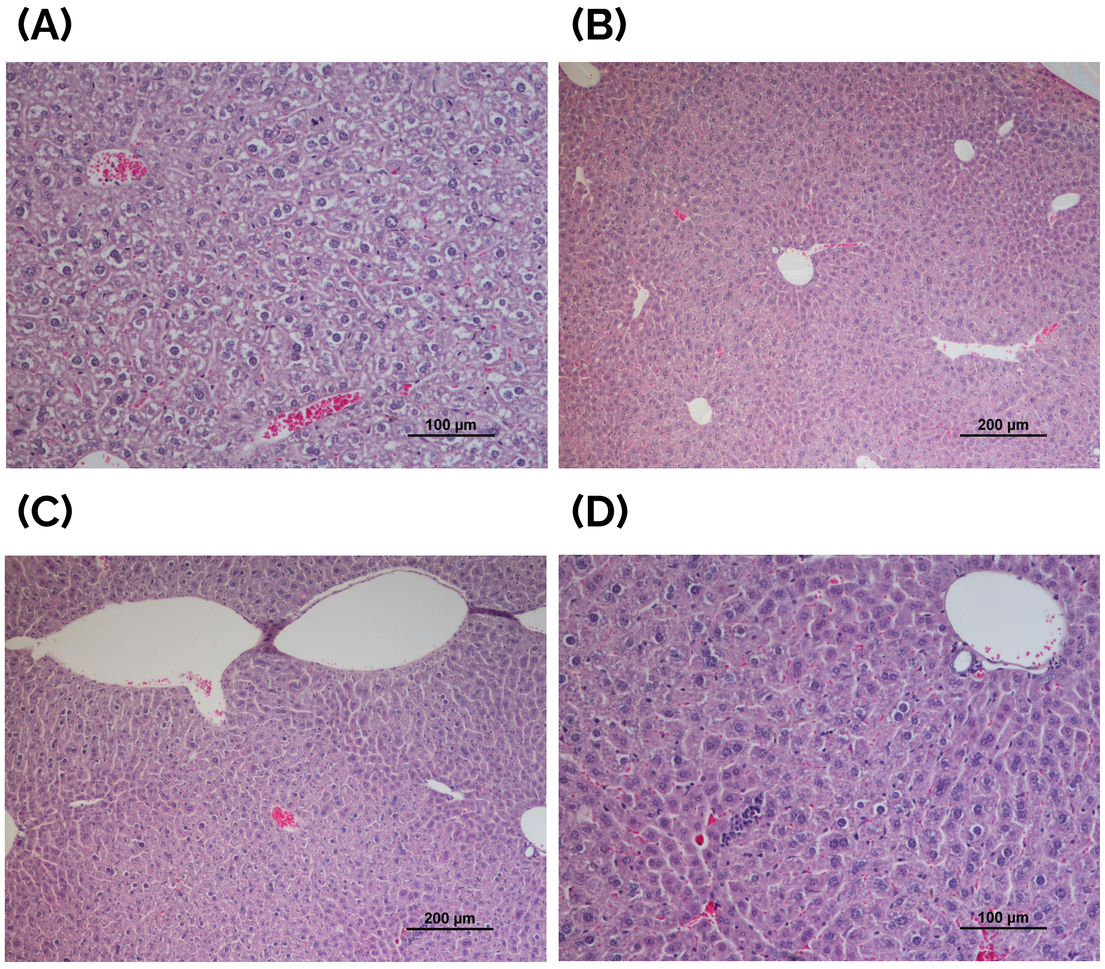

Fig. 2 | Microscopic images (staining with hematoxylin and eosin) of liver sections from the different groups under study. **A)** cellular tumefaction and hydropic changes in a mouse's liver from the control group; **B)** hydropic changes observable only in the centrilobular region in an animal's liver from EE12; **C)** cellular tumefaction and centrilobular hydropic changes in a mouse's liver from EE24; **D)** cellular tumefaction, centrilobular hydropic changes multifocal inflammatory infiltrate in an animal from the EE48.

Concluding remarks

The results suggest that elderberry extract supplementation does not appear to **cause toxicological effects** in kidney or liver structure or function with pathological significance. Further studies should help understand if elderberry has a protective impact on these tissues.

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