



Abstract

# Histopathological Evaluation of Mice's Liver and Kidney after Exposure to an Elderberry Extract <sup>†</sup>

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**Abstract:** Elderberry (*Sambucus nigra* L.) is recognised for its use as a food source, food additive, in nutraceuticals, and in folk medicine. Its berries have a high concentration of 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. The anthocyanin profile was determined using HPLC-DAD-ESI/MS. The study was approved by the Portuguese Veterinary Authorities. A total of 24 FVB/n female mice ( $n = 6/\text{group}$ ) were randomized into four experimental groups: Control, 12 mg/mL EE (EE12), 24 mg/mL (EE24) and 48 mg/mL (EE48). EE was supplemented in drinking water. 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. The predominant anthocyanins detected were cyanidin-3-O-sambubioside-5-O-glucoside and cyanidin-3-O-sambubioside. Histological analysis of the liver showed a significant difference ( $p = 0.036$ ) between Control (80.0%) and EE12 (16.7%), with the Control group's 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. The results suggest that elderberry extract supplementation does not appear to cause toxicological effects in kidney or liver structure or function with pathological significance.

**Keywords:** elderberry; histopathological studies; supplementation; in vivo; natural colorants



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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Portuguese Competent Authority (“DGAV—Direção Geral de Alimentação e Veterinária”, approval No. 10/2013), and reviewed by an Ethics Review Body (“ORBEA—Órgão Responsável pelo Bem-Estar e Ética Animal” under reference 0421/000/000/2014).

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