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## BIOMARKER EVALUATION AND TOXICOLOGICAL STUDY OF ESSENTIAL OILS OF FOENICULUM VULGARE AND CALAMINTHA NEPETA

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**Abstract:** Background: Mediterranean agroforestry systems in Alentejo are characterized by cork and holm oaks and a high diversity of plants with potential medicinal and nutritional interest. Aromatic plants may have an important role in silvo-pastoral sustainability, biodiversity of agrosystems and livestock health. Essential oils (EOs) are important for their antioxidant and antimicrobial properties, but terpenes, their primary constituents, are potentially toxic to herbivores and their toxicity should be evaluated. *Foeniculum vulgare* Mill. (fennel) and *Calamintha nepeta* subsp. *nepeta* (L.) Savi (calamint) are aromatic plants widespread in Mediterranean agrosilvopastoral systems. Previous studies showed that *F. vulgare* and *C. nepeta* EOs have important antioxidant potential and antimicrobial properties, and may contribute to the health and profitability of livestock.

Purpose: The aim of this study was to evaluate the toxicological properties of EOs of *F. vulgare* and *C. nepeta*, widespread in Mediterranean agrosilvopastoral systems and often used as food condiments in Alentejo.

Methods: EOs were obtained from aerial part of plants by hydrodistillation and chemical composition was evaluated by GC-FID. Toxicity of essential oils was evaluated by the estimation of LC<sub>50</sub> in brine shrimp and LD<sub>50</sub> in mice. Oral toxicity assays were performed in mice, based on hippocratic screening during 14 days. Histological analyses and quantification of biomarkers aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase ( $\gamma$ -GT), bilirubin, creatinine and urea were performed for monitoring liver and kidney functions.

Results: The main components of EO of *F. vulgare* were anethole, fenchone and  $\beta$ -myrcene, while EO of *C. nepeta* was rich in isopulegol, isopulegone, 1,8-cineol and (S)-(-)-pulegone. EOs presented high toxicity against *A. salina* ( $200 < LC_{50} < 267 \mu\text{g/mL}$ ) and low toxicity in *Swiss* mice ( $LD_{50} \leq 2000 \text{ mg/kg}$ ). The histological studies of the liver and kidney and the quantification of functional biomarkers showed low toxicity of EOs. Concluding, the EOs of *C. nepeta* and *F. vulgare* showed very low toxicity suggesting their potential use as food supplement. Additionally, our studies point out the importance of the integration of *C. nepeta* and *F. vulgare* in silvopastoral agroforestry systems, contributing to the animal health and profitability of livestock.

**Keywords:** *Foeniculum vulgare*; *Calamintha nepeta*, essential oils, toxicological evaluation, biochemical biomarkers

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