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Occurrence of pyrrolizidine alkaloids in Portuguese teas and herbal infusions: a toxicological assessment using *C. elegans*

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Abstract

Background: Pyrrolizidine alkaloids (PAs) and their *N*-oxide (PANOs) are plant secondary metabolites with recognized hepatotoxic and genotoxic potential, particularly the 1,2-unsaturated forms [1]. Tea and herbal infusions (THI), widely consumed for their known health benefits, are one of the main dietary sources of human exposure to these contaminants [2,3]. **Objective:** This study aimed to detect and quantify PAs/PANOs in THI available in the Portuguese market using a liquid chromatography tandem mass spectrometry (LC-MS/MS) method and to assess the toxicity of selected extracts. **Methods:** Twenty-six samples (chamomile, lemon balm, lemon verbena, green, and black tea) were acquired from the Portuguese market. Samples were extracted by solid-liquid extraction with acidified water at 80°C, followed by solid-phase extraction and analyzed using a Luna® 3µm PFP (2) 100 Å column in the LC-MS/MS system. Based on LC-MS/MS results for PAs occurrence, 4 herbal infusions with total PA concentrations ≥ 20 ng/g were selected for assessing their safety at human-relevant exposure levels. Extracts were prepared using the same infusion procedure and then lyophilized. The *C. elegans* strain DC19 [*bus-5(br19)*] was used to evaluate the effects of the extracts on animal survival and lifespan. Synchronized L1 larvae were exposed to increasing extract concentrations and analyzed after 72 h. Survival was determined by counting live and dead worms, while lifespan was monitored every 2 days by recording mortality. **Results:** Among the 26 samples, 13 contained detectable PAs, of which 5 had total concentrations ≥ 20 ng/g. Herbal infusions showed the highest PA concentrations, ranging from 25.4 to 68.8 ng/g. None of the extracts reduced survival at any tested concentration corresponding to the equivalent consumption of 1–6 cups ($p > 0.05$) [% of surviving animals in all conditions ≥ 95%]. The highest concentration was used to assess lifespan; no significant differences were detected ($p > 0.05$). **Conclusions:** Although PAs/PANOs concentrations were low and no significant effects on *C. elegans* survival or lifespan were observed, the variability among samples highlights the need for monitoring PAs/PANOs in THI. Moreover, the absence of phenotypic alterations at the organismal level does not exclude the possibility of subtle cellular or molecular effects that may not be immediately detectable. Thus, additional cellular, molecular, or biological endpoints are needed to support a more comprehensive risk assessment.

Keywords: tea and herbal infusions; pyrrolizidine alkaloids; LC-MS/MS; *C. elegans*; food safety

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