

## Carboxylesterase and Ethanol Influence Hepatotoxicity of the Areca Nut Alkaloid, Arecoline

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**Objectives:** The areca (betel) nut, popular for its stimulating effects, is one of the most abused substances worldwide. Abundant evidence shows consuming the areca nut (AN) exhibits multiplex toxicity in humans, including oral and hepatic carcinomas. Co-consumption of AN and ethanol entails even more harmful clinical sequelae. We previously identified that combination of ethanol and arecoline in human liver produces an unknown transesterification-derived metabolite called arecaidine ethyl ester (AEE). The objectives of this study were to identify the human enzyme that catalyzes formation of AEE, and compare the toxicity of AEE to known AN alkaloids, arecoline and arecaidine, in a human liver carcinoma cell line (HepG2).

**Experimental Methods:** Kept under simulated physiological conditions, arecoline was incubated with ethanol and human carboxylesterase-1 (CES1) or CES2. The formation of AEE was subsequently measured by high-performance liquid chromatography (HPLC). For cytotoxicity studies, HepG2 cells were plated in 96-well plates in triplicate at a density of  $10^4$  cells/well, and then exposed to increasing concentrations of arecoline, arecaidine, or AEE (1–500 $\mu$ M). After 48 hr incubation, cell death was assessed by live/dead assay and quantitative fluorescence microscopy.

**Results:** CES1 preferentially converts arecoline to AEE only in the presence of ethanol. AEE exhibited dose-dependent toxicity to HepG2 cells 48 hr post-exposure, which was significantly different from controls. AEE displayed significantly more toxicity than arecoline (a known toxin) at 10 $\mu$ M, and trended towards greater cell death than arecoline at other tested concentrations. Unexpectedly, arecaidine exhibited dose-dependent toxicity that was significantly greater than control, but only at 250 and 500 $\mu$ M and was inferior to AEE.

**Conclusion:** We demonstrate for the first time that CES1 is a major driver of arecoline toxification to AEE when ethanol is present. AEE was cytotoxic to HepG2 cells, suggesting it is a plausible player in developing toxicity following AN and ethanol co-consumption.

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