From Neat Compounds to Complex Mixtures:

In Vitro Functional Cardiotoxicity Testing of Botanical Extracts

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Background

Natural products, including botanical dietary supplements, are widely used but have variable chemical compositions due to cultivation, extraction, and processing factors. Ensuring their safety is critical, yet no routine in vitro methods exist for testing these complex mixtures. National Institute of Environmental Health Sciences (NIEHS), and Health and Environmental Sciences Institute (HESI) is evaluating New Approach Methodologies (NAMs) for their applicability to botanical mixtures.

Methods

For the safety endpoints, a battery of well-established cardiotoxicity assays for single chemicals was selected to evaluate their suitability for botanical mixtures. The assay parameters included contraction force, beat rate, duration, upstroke/downstroke slopes, and arrhythmic events, complemented by metabolic and calcium transient analyses. The assays were performed in human iPSC-derived cardiomyocytes (hiPSC-CMs) cultured in a 96-well microphysiological system mimicking native heart tissues and its vasculature, enabling physiologically relevant contractile measurements.

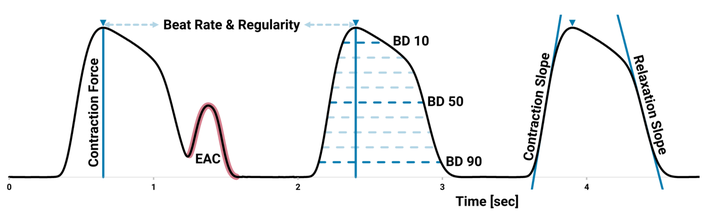


Figure 1: Parameters for the assessment of botanical effects on the contractile properties of hiPSC-CMs.

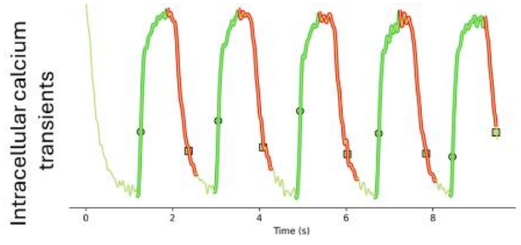


Figure 2: Intracellular calcium transient analysis parameters for cardiomyocyte functional assessment

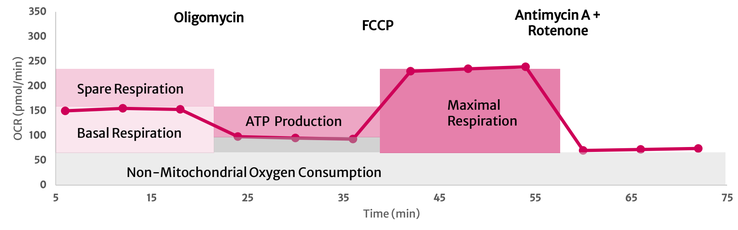
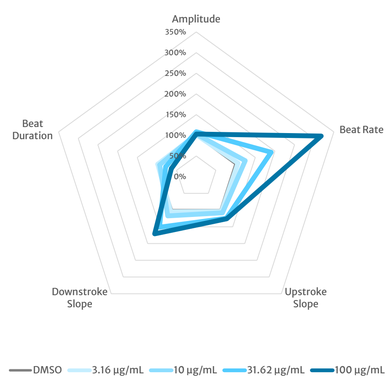


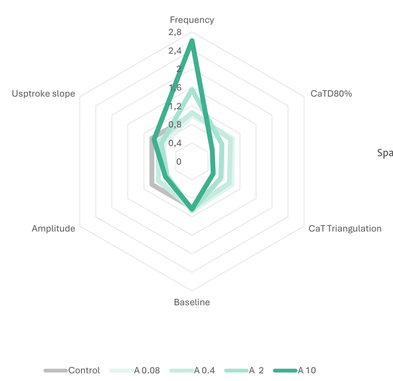
Figure 3: Seahorse respiration assay parameters for the assessment of botanical cardiotoxicity

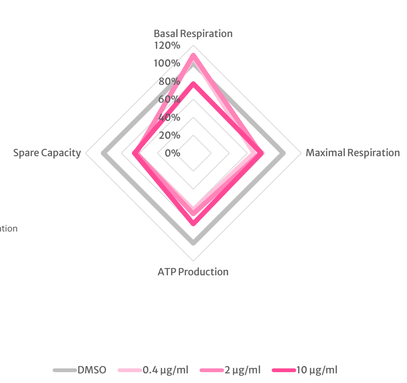
Results

Botanicals with known cardiotoxicity were chemically profiled and tested, while milk thistle and Asian ginseng served as negative controls. Among 16 botanicals, aconite, oleander, goldenseal, and yohimbe showed significant effects on contraction force, beat rate, and duration, aligning with their arrhythmogenic potential. Ashwagandha, kratom, comfrey, and thunder god vine had minimal or no impact. Ephedra transiently increased the beat rate by ~40%, resolving within 24 hours. The findings were substantiated by calcium transient and metabolic analyses. The botanicals with high cardiotoxic risk showed significant impact on the calcium handling of hiPSC-CMs both under acute and chronic administration, while chronic oxygen consumption analysis revealed impact of a broad range of botanicals, indicating the need for refinement of the concentration ranges and other assay parameters.

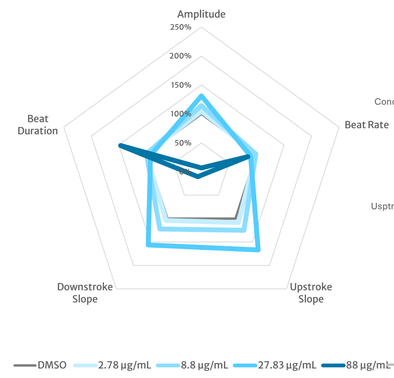
Aconite

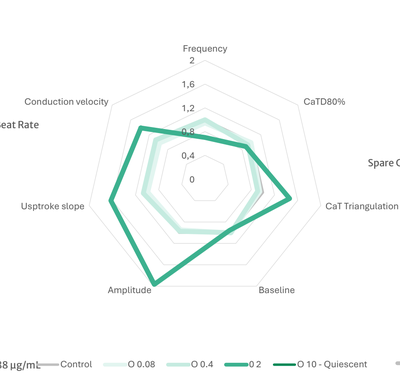


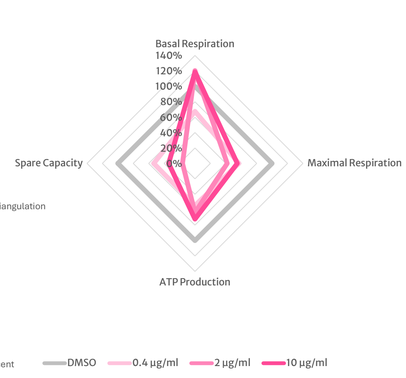




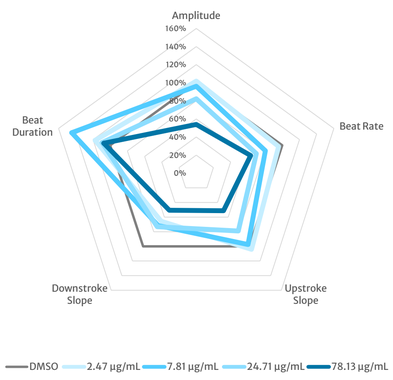
Yohimbe

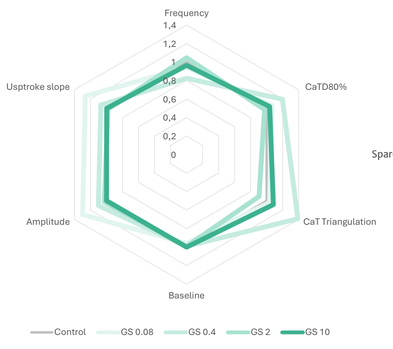


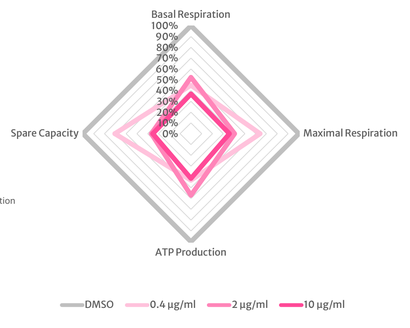




Goldenseal







Ginseng

Milk Thistle

Figure 4: Representative data on the effects of botanicals on hiPSC-CMs. Aconite, Oleander, Glodenseal and Yohimbe have well-characterized effects on cardiomyocytes. Ginseng and Milk Thistle are not known to have effects. **Blue:** Contractile parameters: Contraction force, Beat rate, Upstroke slope, downstroke slope, beat duration. **Green:** Calcium imaging parameters: Frequency, calcium transient duration 80%, calcium transient trangulation, baseline, amplitude, upstroke slope, conduction velocity. **Magenta:** Metabolic parameter: Basal respiration, maximal respiration, ATP production, spare capacity.

Conclusion

The results with human induced pluripotent stem cell-derived cardiomyocytes show that currently applied in vitro assays holds promise for detecting cardiotoxicity in botanical mixtures. These findings suggest that assays currently validated by an international multi-site effort led by HESI and the FDA for single chemicals may also be suitable for cardiotoxicity screening of botanical dietary supplements. Future studies will involve testing a broader range of botanicals and varied preparations (e.g., different solvents) to further understand the scope and limitations of these in vitro tools for cardiotoxicity assessment in complex botanical mixtures.