From neat compounds to complex mixtures: A potential screening strategy for cardiotoxic potential of botanicals

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Background

- Intentional consumption of herbal supplements is increasing.
- Botanical supplements are complex mixtures
- Traditional *in vivo* animal toxicity testing on these complex and variable substances is not always practical/feasible and it is resource intensive.
- The Botanical Safety Consortium (BSC), a public-private partnership, formed by the US FDA, NIEHS, & HESI
- The BSC aims to improve overall botanical products safety by evaluating the suitability of new approach methodologies (NAMs) for botanicals as complex mixtures with cardiotoxicity being a key focus area.

Methods

A battery of cardiotoxicity assays using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs, iCell2 – FujiFilm CDI, USA) currently undergoes evaluation of accuracy in assessing cardiac effects of botanicals as complex mixtures.

- Mitochondrial assessment (Seahorse, JC-1, mitosox)
- Multi electrode arrays electrophysiology
- Optical mapping (action potentials and calcium transients – GCaMPf6)
- Cell viability with cardiac syncytia imaging.
- Direct Contractility

Botanicals with well documented cardiac potential were sourced, characterized, and used across the biological assays

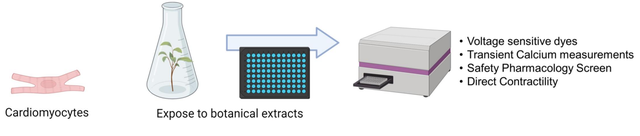

Figure 1: Pipeline to test new approach methodologies to assess botanical products cardiac safety.

Botanical Case Studies

Botanical extracts were distributed to different laboratories for assessment of cardiac effects using hiPSC-CM based assays. The table indicates examples of botanicals assessed for contractility, intracellular calcium changes with optical mapping and mitochondrial superoxide production with mitosox.

Table 1: Botanical extracts, rational for inclusion in the study and known cardiac effects.

Botanical extract	Rationale for inclusion	Expected Cardiotoxicity
Aconite (*Aconitum napellus)*	Known cardiotoxic botanical	Yes
Oleander (*Nerium oleander)*	Known cardiotoxic botanical	Yes
Asian ginseng root (*Panax ginseng)*	Large body of evidence pointing to safety	No
Ephedra (*Ephedra sinica)*	Evidence of cardiotoxicity	Yes
Milk Thistle Seed (*Silybum marianum)*	Large body of evidence pointing to safety	No

Contractile parameter assessment

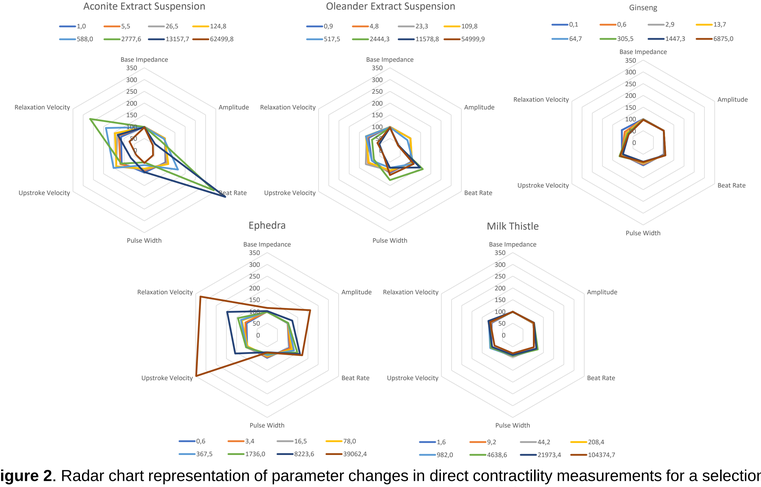

Figure 2. Radar chart representation of parameter changes in direct contractility measurements for a selection of botanical extracts. Values are given in percent of DMSO control. 8 cumulative dose concentrations were added in each well and the corresponding acute (5 min) responses were acquired.

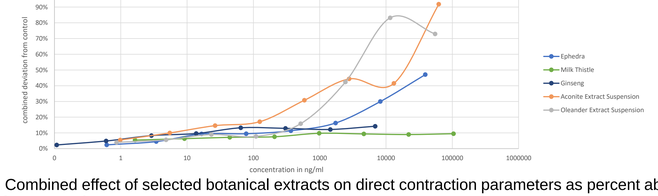

Figure 3. Combined effect of selected botanical extracts on direct contraction parameters as percent absolute deviation from control.

Intracellular calcium transients optical mapping and mitochondrial superoxide assessment

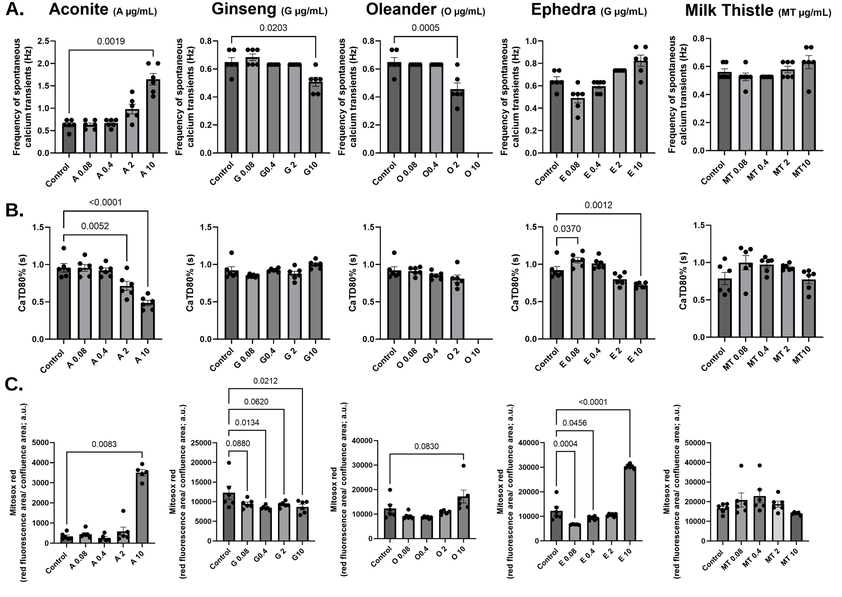

Figure 4. Optical mapping of intracellular calcium transients after exposure to botanical extracts at 0, 0.08, 0.4, 2 and 10µg/mL. **(A)** Acute effects (1 hour) of botanical extracts on the frequency of spontaneous intracellular calcium release. **(B)** Acute effects of botanical extracts on intracellular calcium transient duration at 80% of calcium reuptake into the sarcoplasmatic reticulum. **(C)** Assessment of chronic exposure of hiPSC-CMs to different concentration of botanical extracts. Brackets indicates p<0.1.

Conclusions & Future Directions

Botanical extract presented the expected effects on all assays and results indicate that, many assays currently available for single chemicals may be suitable for assessment of complex botanical mixtures and assays may be selected to match a context-of-use of the botanical product. As future directions we will expand the number of tested botanical extracts and use results in ADME models to better understand results and feed a multiple evidence streams model (*in silico* and *in vitro*) in a Weight of Evidence analysis to provide accurate safety evaluation.

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