From Neat Compounds to Complex Mixtures: In Vitro Functional Cardiotoxicity Testing of Botanical Extracts

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Background

Natural botanical supplements are widely used but have variable compositions due to cultivation, extraction, and processing factors. Ensuring their safety is crucial, yet no standard *in vitro* testing exists. Toxicity data is limited, and few screening tools have been evaluated. In a global effort by the FDA, NIEHS and HESI, *in vitro* methods from single-chemical toxicity testing were assessed for their relevance to botanical mixtures.

Methods

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs, iCell Cardiomyocytes[2], Fujifilm Cellular Dynamics) were treated with a total of 16 botanical extracts for 20 min to 24 h at four different concentrations and analyzed for their contractile properties using the FLEXcyte 96 system. For the assessment of contractile properties, the cardiomyocytes are cultured on ultra-thin (4 µm) silicone membranes, mimicking the mechanical environment of native human heart tissue with an elastic modulus of 33 kPa. Deflected by the weight of the cell culture medium, rhythmic contractions of the cells lift the membranes upwards, quantified by capacitive distance sensing. Parameters analyzed are force of contraction, beat rate, beat duration, upstroke slope and downstroke slope (Figure 1).

Results

From the 16 botanicals tested, aconite, oleander, goldenseal and yohimbe showed the largest effect sizes in accordance with their anticipated effects described in the literature (Figure 2). Aconite increased the beat rate by over 200% of control while decreasing the total beat duration. In contrast, yohimbe and oleander decreased the beat rate to approx. 50% of control, while increasing the beat duration, indicating arrhythmogenic potential. Ashwagandha, kratom, comfrey and thunder god vine showed little or no effect at all concentrations. Green tea (decaffeinated) had a strong dose-dependent negative effect on contraction force and beat rate (-20% of control, each) after 24 h of incubation, while prolonging the beat duration by 20%. This finding might be due to the high concentrations applied in this experimental setup (2.47 – 78.13 mg/L), exceeding blood concentrations achieved by regular consumption patterns.

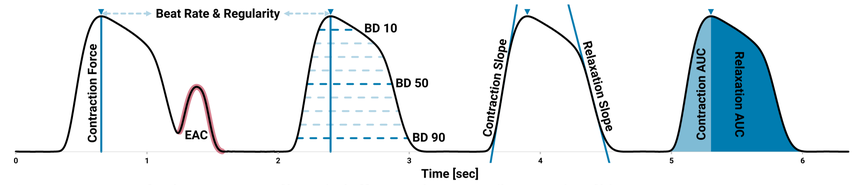

Figure 1: Parameters for the assessment of botanical effects on the contractile properties of hiPSC-CMs.

Table 1: Summary of the effects of the 16 botanicals on the amplitude (contraction force), beat rate and beat duration after 20 min and 24 h.

	Effect size 20 min			Effect size 24 h			
Extract	Amplitude	Rate	Duration	Amplitude	Rate	Duration	
Aconite	9%	218%	-37%	-23%	177%	-37%	acute & chronic
Oleander	-93%	-16%	47%	-100%	-100%	-100%	
Goldenseal	-46%	-37%	45%	-100%	-100%	-100%	
Yohimbe	-38%	-33%	32%	-43%	-51%	20%	
Milk Thistle	-13%	-8%	23%	-22%	-22%	34%	
Green tea	-14%	10%	8%	-21%	-24%	17%	chronic
Ginseng	4%	-6%	26%	9%	-12%	23%	acute & chronic
Blue Cohosh	3%	15%	11%	11%	2%	35%	
Ephedra	3%	37%	-16%	2%	11%	7%	acute
Usnea lichen	-5%	-2%	-7%	-15%	-11%	-7%	low/no effect
Kava	-5%	-5%	6%	-8%	-6%	16%	
Aristolochia fangchi	2%	9%	6%	-6%	13%	3%	
Thunder god vine	2%	-1%	8%	-6%	2%	16%	
Ashwagandha	5%	-2%	10%	6%	-3%	8%	
Kratom	-4%	-3%	-5%	-5%	-4%	11%	
Comfrey	2%	-1%	8%	6%	5%	5%	

Figure 2: Representative data on the effects of botanicals on hiPSC-CMs. Red: Aconite, oleander, goldenseal and yohimbe had strong effects on all contractile parameters. Green: Ashwagandha and comfrey had little or no effect. Blue: Green Tea affected the contractile parameters only at the highest concentration after 20 min, while the effect size increased at all concentrations after 24 h.

Conclusion

The results with human-induced pluripotent stem cell-derived cardiomyocytes show that the assay 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.