AN ESSENTIAL OIL BLEND MODULATES NEURONAL HEALTH RESPONSES AFTER CHEMICAL STRESS

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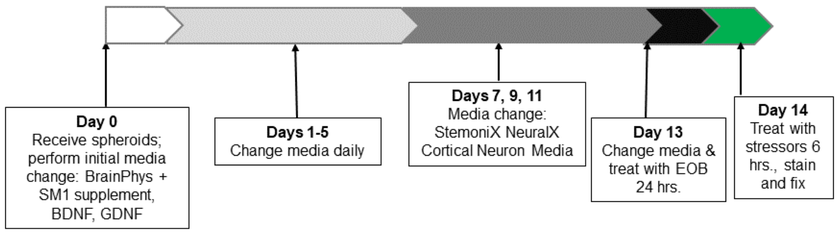
Overview

Despite growing scientific evidence that essential oils possess therapeutic benefits, research on their activities in complex human disease models is scarce. We analyzed the biological activities of essential oils (EO) and blends of EO and turmeric extracts (EOB; provided by dōTERRA) in StemoniX microBrain 3-D neurospheres containing iPSC-derived human cortical neurons (both glutamatergic and GABAergic) and astrocytes from a single source. We used our established high-content imaging assays for neuronal health, including neuronal viability, mitochondrial function, and neurite outgrowth. iPSC-derived neurons were cultured, treated with EO/EOB, treated with a stressor (FCCP or tunicamycin), fixed, and then stained for imaging to measure changes in neuronal features impacted by stressors and improvements induced by EO/EOB treatment. The stressors impacted neuronal health in most measures, particularly viability levels and mitochondrial function, and treatment with EO/EOB significantly reversed many of these deficits. This study suggests that EOB may have a protective effect on the overall health of human iPSC-derived neurons in response to environmental stress.

Neurosphere Culture and Experimental Set-up



Overview: Neurospheres were cultured for 12 days and then treated with either DMSO (control) or EO/EOB on day 13. Neurospheres were treated with stressors for 6 hours before stained and fixed for imaging.

Treatment with EO/EOB: MicroBrains were treated with 3 doses of essential oil (dōTERRA R&D lot#GA1806017), 95% turmeric extract (dōTERRA lot#201803090049), or combinations of both for 24 hours prior to addition of stressor compounds.

EO doses tested: 0.001% (low), 0.0033% (med.), and 0.01% (high).
Turmeric extract doses tested: 1uM, 5uM, and 25uM
EOB doses tested: 0.001% EO + 1uM extract (low), 0.0033% EO + 5uM extract (med.), and 0.01% EO + 25uM extract (high).
0.1% DMSO was tested as vehicle control. N=4 replicate wells for all conditions.

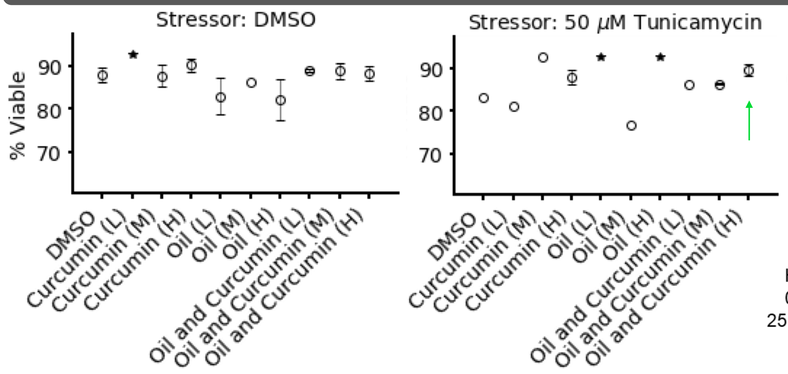
Treatment with Stressors: Either FCCP or tunicamycin were added to the microBrains in 3-point, dose-response format for 6 hours prior to fixation. Doses tested were 1uM (low), 10uM (med.), and 50uM (high).

Staining: Prior to fixation, microBrains were stained with MitoTracker Red CMXRos to assess mitochondrial function and DRAQ7 to assess cell viability. MicroBrains were stained with an anti-TUJ1/β-3-tubulin antibody to quantify neurite area. DRAQ5 stain was also applied to enhance nuclear staining.

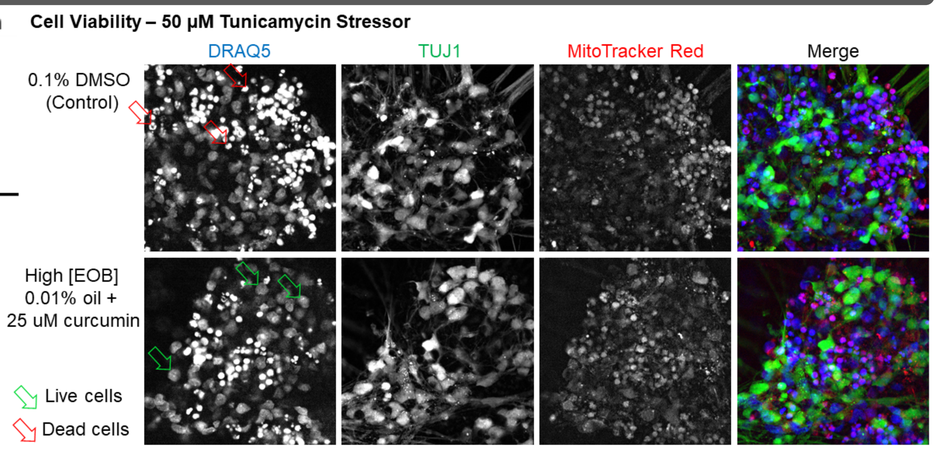
Analysis: Automated quantitative analysis of viable cells was conducted using the Thermo CX7 HCS instrumentation (20x magnification) and analysis suite. Readouts for cell viability, mitochondrial function, and neurite outgrowth were calculated and compared across treatment conditions.

Representative images of neurons protected by EO/EOB treatments are shown in the results.

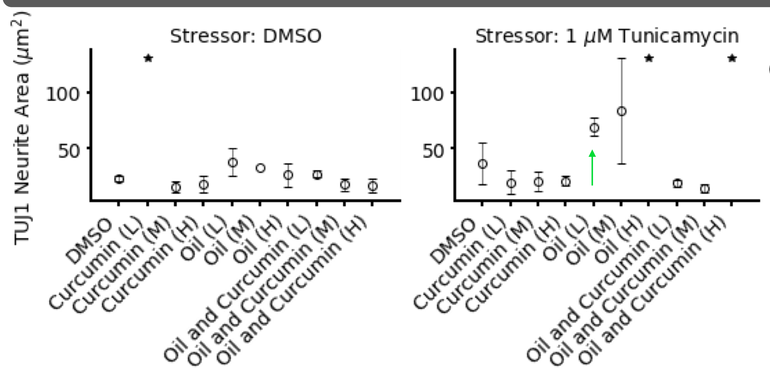
Cell Viability is Protected by an Essential Oil Blend (EOB)



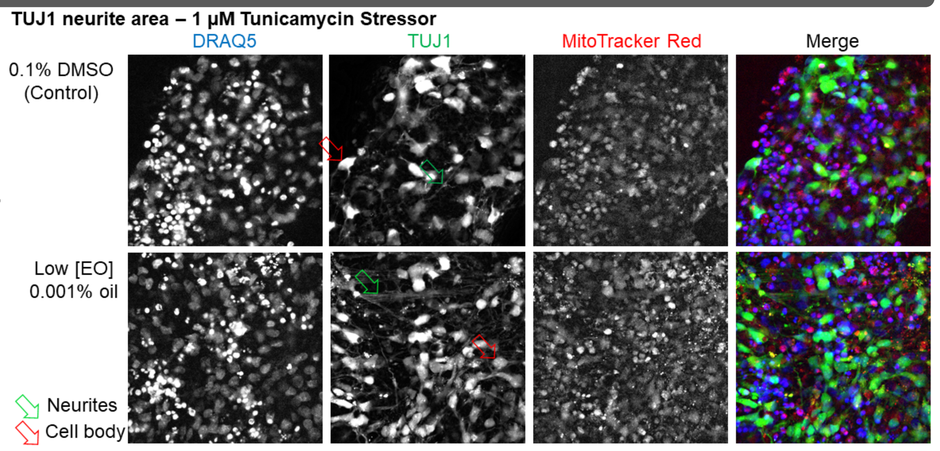
Cell viability was protected by treating with high dose of EOB prior to exposure to 50μM tunicamycin.



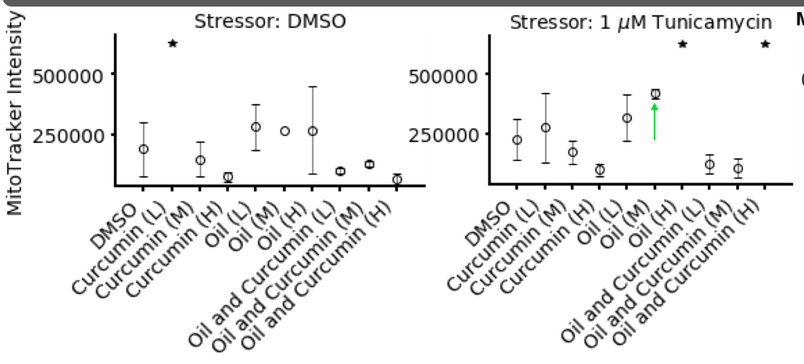
EOB Protects Neurites from Chemical Stress Treatment



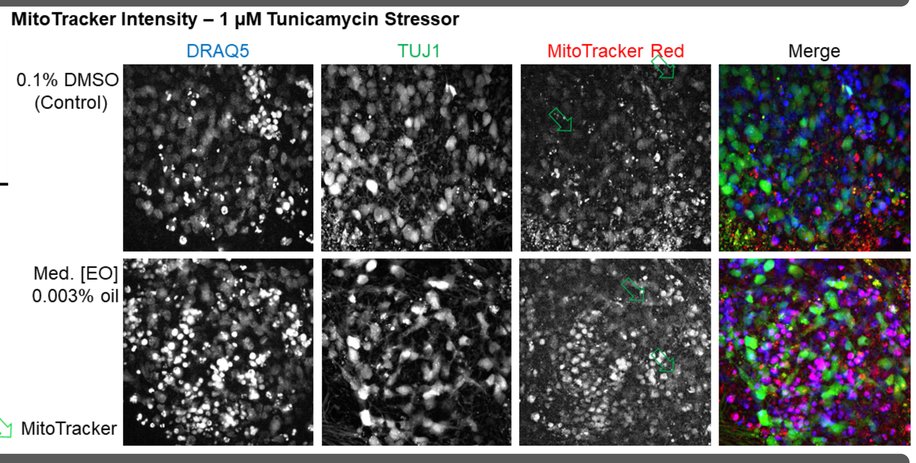
Neurite outgrowth was protected by treating with 0.001% EO prior to exposure to 1μM tunicamycin.



EOB Blocks Effect of Chemical Stress Treatment



Mitochondrial activity was protected by treating with 0.003% EO prior to exposure to 1μM tunicamycin.



Summary: EOB's May Protect iPSC-Neurons from Environmental Stress

- dōTERRA EO and EOB did not significantly affect cell viability, mitochondrial activity, nor neurite outgrowth in microBrains in the absence of stressors.
- EO/EOB may have neuroprotectant properties against environmental stressors.
- EO/EOB did not significantly change microBrains' response to FCCP treatment.
- Med. and high [EO] protected cell viability from high [tunicamycin].
- Low, med., and high [EOB] improved cell viability in response to treatment with high [tunicamycin].
- Low [EO] protected neurite outgrowth from treatment with low [tunicamycin].
- Med. [EO] protected mitochondrial activity from treatment with low and high [tunicamycin].