Brain Cell Study

Facility: DV Biologics states, (DVB)

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Solution used: Earth Science Tech, Inc. (ETST) Full Spectrum Crude/Unfiltered Cannabinoids derived naturally procuring from industrial hemp plants.

Study type: In Vivo Trial

Report and Effects of ETST's High Grade Full Spectrum Cannabinoids on Brain Cells

The Brain: When fat (lipids) from food is digested, it is later broken down into fatty acid molecules. The brain then uses these for raw materials to assemble the special types of lipids it incorporates into its cell membranes. Two-thirds of the brain is composed of lipids. The vast majority of the different cell types in the brain require lipids for correct transmission of signals, providing energy and structural support to our cell membranes.

Lipid Peroxidation: Cells in the body and particularly in the brain are under constant attack by free radicals and oxidative stress. There are protective mechanisms that help deter this normal damage that occurs; however, with aging the protective mechanism declines substantially and degeneration of the brain occurs.

Lipid peroxidation is the degradation of lipids that occurs as a result of oxidative damage and is a useful marker for oxidative stress. Lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of end products such as malondialdehyde (MDA).

In this experiment, lipid peroxidation is determined by the reaction of MDA with thiobarbituric acid (TBA) to form a fluorometric complex, proportional to the amount of MDA present, after induction of peroxidation with hydrogen peroxide.

A. Experimental procedure:

A.1. Two 24-well plates were coated with Matrigel for 1 hour at 27° Celsius.

A.2. The plates were seeded with human neural cells at a density of 2×10^4 cells/cm^2. The cells were grown in neural media supplemented with 20 ng/ml of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).

A.3. Media was changed every 2-3 days.

A.4. Full spectrum cannabinoids Neural-Growth media was prepared by diluting the full spectrum cannabinoids in dimethyl sulfoxide (DMSO). The suspension was filtered with a 0.22 micron filter. The full spectrum cannabinoids suspension was then diluted in N-GRO media to contain the following concentrations of CBD 10 μM, 5 μM, 2.5 μM, and 1 μM CBD.

A.5. After the cells reached 80-90% confluency, base media was replaced with media containing full spectrum cannabinoids or control media; the controls were media only, media with DMSO 1:100, and media with hydrogen peroxide (H_2O_2) (H_2O_2 was added during induction phase).

A.6. The cells were incubated under experimental conditions for 48 hours in media containing full spectrum cannabinoids.

A.7. After 48 hours, lipid peroxidation was induced by addition of H_2O_2 at concentration of 100 µM to certain wells in duplicates. H_2O_2 was added according to the following protocol:

Well ID	A	B	C	D	E	F
1	Media only (control)	Media +DMSO (control)	1 µM CBD + H_2O_2	Media+2.5µM CBD only	5 µM CBD + H_2O_2	Media+10µM CBD only
2						
3	Media + H_2O_2	Media+1µM CBD only	2.5 µM CBD + H_2O_2	Media+5µM CBD only	10 µM CBD + H_2O_2	
4						

A.8. The cells were incubated with H_2O_2 for 120 minutes at 37° Celsius.

A.9. Media was aspirated and the cells were lysed.

A.10. Total protein was precipitated, and reaction to form MDA-TBA adduct was performed. The reactions were placed in a 96-well plate.

A.11. MDA fluorometric detection was done by measuring fluorescence intensity (excitation 532 nm/ emission 553 nm) of samples and standard curve.

A.12. Relative lipid peroxidation was measured by subtracting blank value from all readings.

B. Results:
Figure legend: ETST's High Grade Hemp Full Spectrum Cannabinoids is protective against lipid peroxidation in human brain cells *in vitro*. Human brain cells were incubated with media (control), hydrogen peroxide (H_2O_2) to cause oxidative stress, DMSO which is the solvent to dilute full spectrum cannabinoids in media and different concentrations of cannabinoids with and without H_2O_2. Error bar = Standard deviation.

When brain cells are incubated with H_2O_2 by itself lipid peroxidation occurs substantially as seen in the graph above. Lipid peroxidation as measured my MDA present is seen to reach greater than 2.5 nM as compared to controls of media and DMSO alone which register 0.5 nM or less. When human brain cells are treated with ETST's High Grade Full Spectrum Cannabinoids in concentrations varying from 1 µM to 10 µM prior to the addition of H_2O_2 (prophylactically), human brain cells are protected against the damaging effects of lipid peroxidation.

C. Summary:
Lipid peroxidation is damaging to human brain cells. ETST's High Grade Full Spectrum Cannabinoids in varying concentrations rescues the damaging effects of lipid peroxidation to human brain cells. The data suggest that ETST's High Grade Full Spectrum Cannabinoids may serve as a neuroprotectant to brain cells. Further studies on other oxidative stress and free radical pathways are needed in order to support and extend this data.