REVIVIFY GEL ATTENUATES HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS (HBMEC) FROM HYPOXIA INDUCED DISRUPTION OF BLOOD BRAIN BARRIER (BBB) PERMEABILITY IN A IN VITRO MODEL

Syeda H. Afroze, PhD[1,2]; Ahmed F. Pantho BS[1,2]; Liaquat Hossain, M.Pharm[3]; Thomas J. Kuehl, PhD[1,2]; M. Nasir Uddin, PhD, FAHA[1,2,3,5*]

[1]Orion Institute for Translational Medicine and [2]Emergent Biotechnologies LLC, Temple, Texas 76504; [3]Advance Pharmaceutical Inc., 895 Waverly Ave, Holtsville, NY 11742;**

[4]Texas A&M University School of Medicine, Texas

***** Advance Pharmaceutical Inc. has patent for the REVIVIFY GEL***

BACKGROUND AND OBJECTIVE

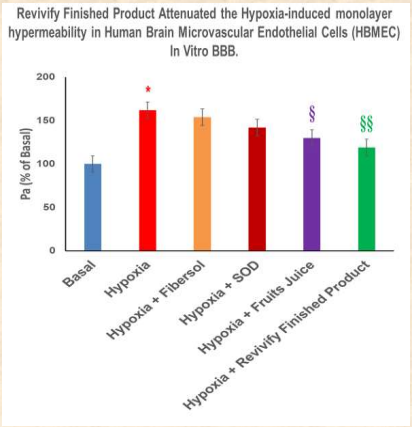
Accumulating data suggests that oxidative stress and mitochondrial damage are involved in the pathogenesis of neurodegenerative disorders including Parkinson Disease [PD], Multiple Sclerosis[MS], Alzheimer' s Disease[AD], and many others. Brain uses about 20% of oxygen consumption, thus high producer of reactive oxygen species [ROS]. Also, brain cell membrane composed of more unsaturated fatty acids [M UFA and PUFA], thus more prone to lipid auto-oxidation due to ROS. REVIVIFY GEL, addresses instant reduction of oxidative stress from multi-dimensional pathways and resulted an immediate effect induced by the disease symptoms. The purpose of the study is to evaluate whether revivify gel attenuates human brain microvascular endothelial cells (HBMEC) from oxidative damage. ***The objective of the study is to evaluate whether Revivify gel attenuates the hypoxia induced disruption of HBMEC Monolayer Permeability.***

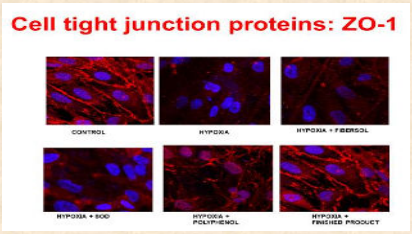
STUDY DESIGN

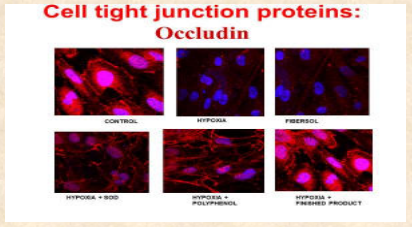
- Human brain microvascular endothelial cells (HBMEC) were seeded on 6 well plates in hypoxia condition. Prior to treatment, cells were incubated in serum free media for 24 hours. Cells will be treated with following agents: 1. Superoxide Dismutase only; 2. Prebiotic fiber only; 3. Fruit juice only; 4. superoxide Dismutase + Prebiotic fiber + Fruit juice (Combination); 5. Negative Control: Cell culture media for 48 hours.

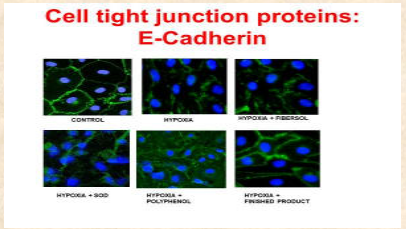
- The monolayer permeability study was performed by a method described previously in hypoxia condition and pretreatment with revivify. HBMEC were grown on poly-L-lysine glass chamber slides. The cells were treated with the treatment conditions mentioned above. After hypoxia condition, cells were washed in PBS and fixed in 4% paraformaldehyde.

- After repeated washing steps, Triton X-100 treatment, and blocking for nonspecific binding, cells were incubated with a primary antibody for ZO-1, Occludin, Claudin-1 or E-Cadherin (Invitrogen) at 4° C overnight. Cells were washed in PBS and exposed to an FITC-conjugated secondary antibody for 1 h. After repeated washing steps, the cells were mounted in an antifade mounting medium that contained the nuclear stain DAPI (Invitrogen, Eugene, OR). Cells were observed under an Olympus FluoView FV 300 confocal laser-scanning microscope with appropriate filters for visualizing FITC and DAPI.









Figures. Immunofluorescence images of endothelial cell tight junction proteins: ZO-1, Occludin and E-Cadherin in HBMEC. CONTROL (No treatment) cells show intact tight junctions evidenced by the strong and continuous presence of ZO-1, Occludin (red color for two) and E-Cadherin (green color) at the junctions.

HOPOXIA: Disruption of the tight junction proteins

HYPOXIA + FIBERSOL: No Attenuation of tight junction proteins

HYPOXIA + SOD: Partial Attenuation of tight junction proteins

HYPOXIA + POLYPHENOL: Attenuation of tight junction proteins

HYPOXIA + FINISHED PRODUCT: Attenuation of tight junction proteins

RESULTS

- **HBMEC monolayer permeability was significantly increased in hypoxic condition. Revivify significantly attenuated the hyperpermeability induced by hypoxia.**
- **Revivify Finished Product Attenuated the Hypoxia-induced monolayer hypermeability in Human Brain Microvascular Endothelial Cells (HBMEC) In Vitro BBB.**
- **Immunofluorescence images of endothelial cell tight junction proteins: ZO-1, Occludin and E-Cadherin in HBMEC.**
- **CONTROL (No treatment) cells show intact tight junctions evidenced by the strong and continuous presence of ZO-1, Occludin and E-Cadherin at the junctions. HOPOXIA: Disruption of the tight junction proteins; HYPOXIA + FIBERSOL: No Attenuation of tight junction proteins; HYPOXIA + SOD: Partial Attenuation of tight junction proteins; HYPOXIA + POLYPHENOL: Attenuation of tight junction proteins; HYPOXIA + FINISHED PRODUCT: Attenuation of tight junction proteins.**

CONCLUSIONS/PERSPECTIVES:

- REVIVIFY GEL; Pertaining to PD, it can improve motor activity, muscle stiffness, and overall body response with less exhaustion.
- For AD, it may improve the memory response, coordination with surrounding atmosphere. As others, it can improve focus, concentration, and alertness, which may be beneficial to people with learning disability, people with autistic problem, people with mental exhaustion, and can benefit to the people who needs study focus, or job associated with high concentration.
- The pre-biotic soluble corn fiber encompasses the healthy gut-echo-system where the modulation of beneficiary microbes influences various positive neurological effect. The gut-brain bi-directional axis can relate instant neuro-responses.
- Thus, REVIVIFY PRO-VITALITY GEL is unique and exert prompt responses towards neuro disease induced symptoms in PD, MS, AD and other conditions.