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Researching life-changing solutions

ASHWITH™

STRESS-REDUCING COGNITION AND PERFORMANCE TECHNOLOGY

ASHWITH™ BENEFITS

- Helps reduce stress and anxiety
- Helps enhance memory and cognition
- Helps support healthy weight management
- Helps promote muscle strength, size and recovery
- Helps promote sexual function in men and women
- Helps maintain healthy testosterone in men
- Helps enhance cardiorespiratory endurance
- Helps maintain normal thyroid

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ASHWITH™ RESEARCH SUMMARY

Comparative efficacy analysis of Biologic's ASHWITH™ ashwagandha (Biologic's engineered ashwagandha root extract with augmented active withanolide proportions) with non-branded and branded (Sensoril) ashwagandha extract.

1. IN VITRO ANTIOXIDANT ACTIVITY ASSAY

Antioxidant activity of various ashwagandha samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH is a stable free radical which produces a violet solution in ethanol and upon reduction by antioxidant compound produces light yellow to a colorless solution.

METHODS:

For comparative anti-oxidant activity analysis of Biologic's ASHWITH™, non-branded and branded (Sensoril) ashwagandha samples, two different dilutions (100 µg/ml and 1000 µg/ml) of each sample prepared in ethanol and 500 µl of each sample dilution mixed with 500 µl of 0.04mg/ml DPPH (Sigma #D9132-1G) ethanolic solution. Gallic acid and sucrose used as positive and negative controls, respectively. All samples and controls incubated for at least 30 min at room temperature in the dark followed by absorbance (Abs) reading at 517nm using a spectrophotometer. Percentage anti-oxidant activity was calculated using the formula: 100-(AbsSample-AbsBlank/AbsControlX100) where AbsSample=Sample dilution+DPPH solution; AbsBlank=Sample dilution+DPPH solvent; AbsControl=Sample solvent+DPPH solution.

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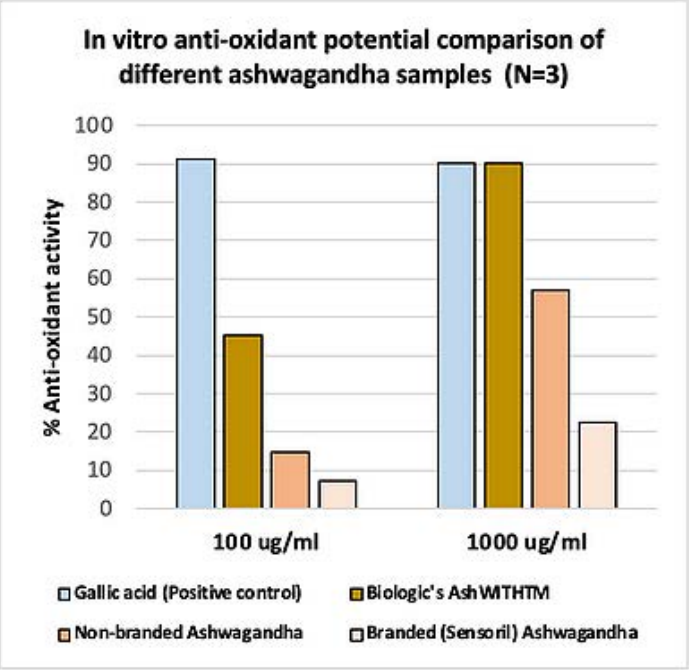
RESULTS:

Biologic's ASHWITH™ ashwagandha showed significantly higher antioxidant activity when compared to non-branded and branded (Sensoril) ashwagandha extract.



Control (DPPH only) | Gallic acid (Positive Control) | Sucrose (Negative Control) | Biologic's AshWITH™ | Non-branded Ash | Branded (Sensoril) Ash

Higher the anti-oxidant potential of the compound more is the discoloration of purple DPPH solution.

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2. CELL VIABILITY ASSAY

MTT based cell viability assay was performed to compare cytoprotection efficacy of various ashwagandha samples against oxidative stress-induced cellular damage.

METHODS:

Hydrogen peroxide (H2O2) (IC50 0.4mm) was used to induce oxidative stress in cultured HEK293 (Human Embryonic Kidney) cells (Fig. 1). Approximately, 8000 HEK293 cells were seeded per well of a 96 well plate in 100ul DMEM and 10% FBS media. Cells were allowed to adhere overnight followed by treatment as follows:

1. Control (DMSO)

2. H2O2 (0.4mM) only

3. H2O2 along with Biologic's ASHWITH™ (15 µg/ml),

4. H2O2 along with Non-branded ashwagandha (15 µg/ml) and

5. H2O2 along with branded (Sensoril) ashwagandha (15 µg/ml) for 22hr.

MTT assay was performed as per standard protocol and absorbance of formazan crystals dissolved in DMSO measured at 540nm using SpectraMax i3X plate reader. The percentage cell viability in the above experimental groups was calculated as follows: [(AbsSample-AbsBlank/AbsControl-AbsBlank)X 100].

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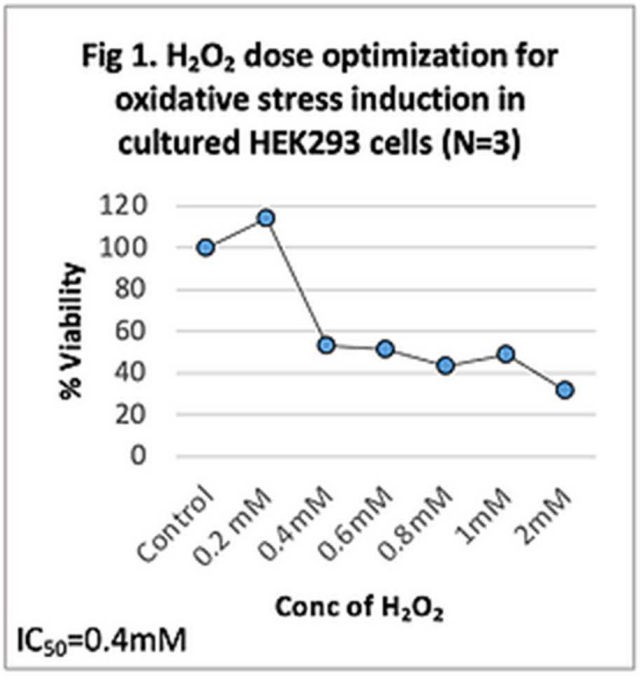
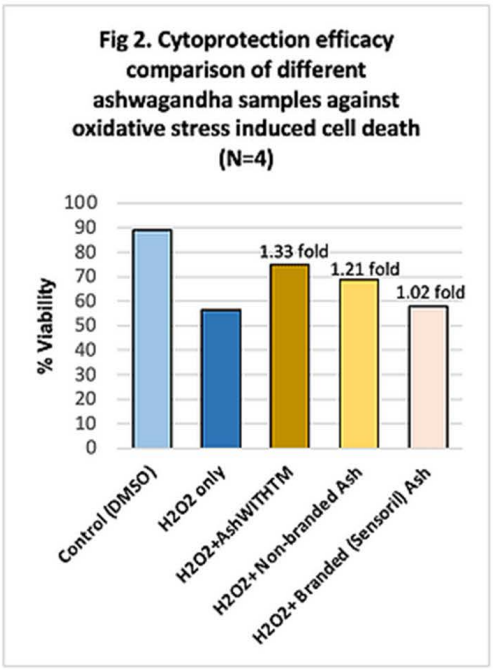
RESULTS:

ASHWITH™ ashwagandha co-treated cells showed better cytoprotection efficacy (1.33 fold) in comparison to non-branded (1.21 fold) and branded (Sensoril) (1.02 fold) ashwagandha co-treated cells (Fig. 2).





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3. REACTIVE OXYGEN SPECIES (ROS) LEVEL MEASUREMENT

Reactive Oxygen Species (ROS) are a natural byproduct of aerobic respiration that are crucial for many cellular signaling pathways. However, elevation in ROS level due to imbalance between generation and detoxification - a condition termed as oxidative stress - leads to cell and tissue damage and has been implicated in the development of various pathological conditions such as premature aging, cancer, atherosclerosis, vascular diseases, etc.

METHODS:

ROS lowering potential of different ashwagandha samples was compared using DCFDA cellular ROS assay kit (Abcam ab113851). Two different compounds were used such as hydrogen peroxide (H2O2) and Sodium arsenite (SA) to induce oxidative stress by increasing cellular ROS in HEK293 cells. TBHP (tert-Butyl hydroperoxide) used as a positive control for ROS generation.

RESULTS:

Biologic's ASHWITH™ ashwagandha co-treated cells showed higher potential to reduce intracellular ROS level caused by both H2O2 (Fig. 1) and SA (Fig. 2) as compared to non-branded and branded (Sensoril) ashwagandha co-treated cells.

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Fig 1.Comparative ROS level in different ashwgandha treated cells exposed to hydrogen peroxide (N=3)

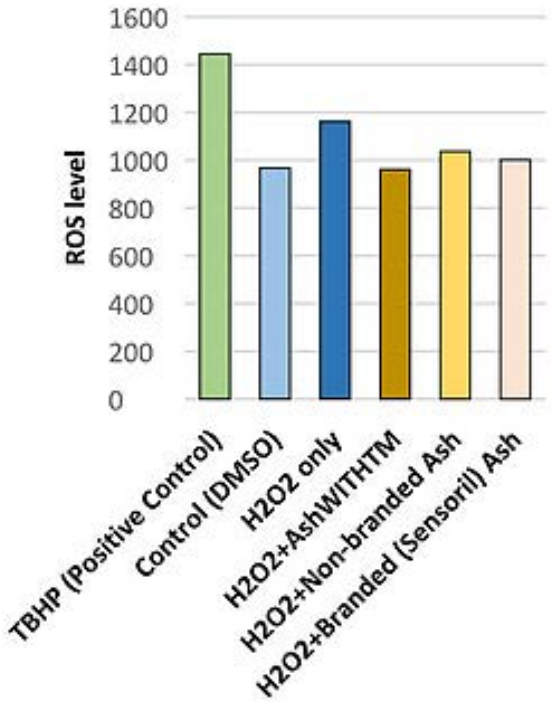
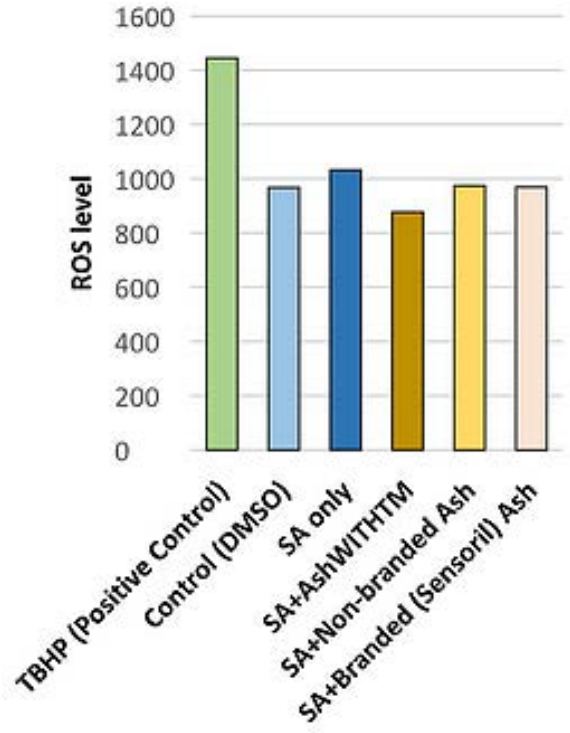


Fig 2. Comparative ROS level in different ashwgandha treated cells exposed to Sodium arsenite (N=3)



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4. EXPRESSION OF INTRACELLULAR ANTI-OXIDANT ENZYME GLUTATHIONE PEROXIDASE

Glutathione Peroxidase (GPx) is a major intracellular anti-oxidant enzyme whose main biological function is to protect the organism from oxidative damage. GPx was first identified in 1957 as an enzyme that protects red blood cells against hydrogen peroxide (H2O2). GPx catalyzes the conversion of H2O2 in to H2 and O2 via oxidation of reduced GSH into its disulfide form (GSSG).

METHODS:

Intracellular GPx1 level was compared in oxidative stress-induced HEK293 cells co-treated with different ashwagandha samples using the semi-quantitative dot blot method. Approximately, 10,000 HEK293 cells were seeded per well of 6-well plates and cultured until cells were 60-70% confluent. Then, cells were treated as follows:

1. Control (DMSO)

2. H2O2 (0.4mM) only

3. H2O2 along with Biologic's ASHWITH™ (15 µg/ml),

4. H2O2 along with Non-branded ashwagandha (15 µg/ml) and

5. H2O2 along with branded ashwagandha (15 µg/ml) for 22hr.

Cells were harvested, total protein quantified and 2µg protein per sample spotted onto nitrocellulose membrane and probed with anti-GPX1 antibody (Abcam #ab22604).

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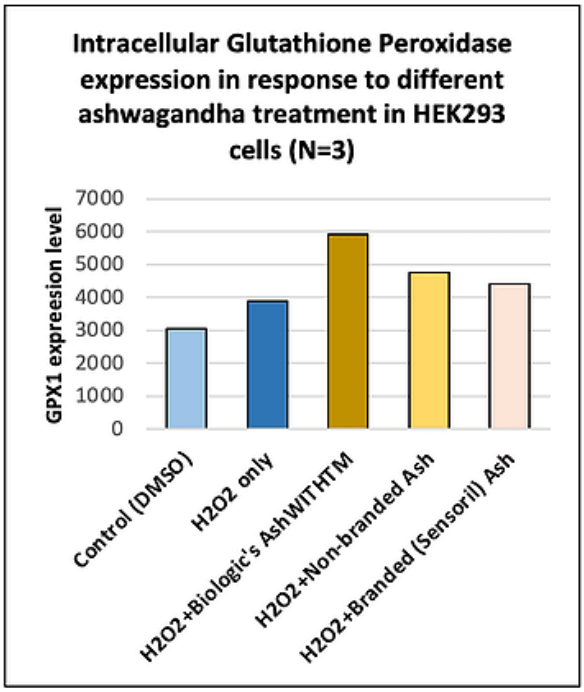
Dot blot membranes were imaged using BioRad's Fluor-S Max multi imager and spot intensity quantified using Quantity One software.

RESULTS:

Ashwagandha co-treated cells showed up-regulation in GPX1 expression as compared to H2O2 only group with highest GPX1 expression observed in Biologic's ASHWITH™ co-treated cells when compared to non-branded and branded (Sensoril) ashwagandha co-treated cells. An increase in GPX1 enzyme expression was possibly due to cellular recovery by increasing resistance to oxidative stress.



Statements have not been evaluated by the Food and Drug Administration.
Product is not intended to diagnose, treat, cure, or prevent any disease. Results will vary.

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