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Impact of the Biofield Energy Treated Proprietary Novel Formulation on Memory and Cognition Function in Neuronal Cell

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Abstract

The current study was investigated for the *in vitro* cognitive effect of the Consciousness Energy Healing (The Trivedi Effect®) Treated novel proprietary test formulation in terms of acetylcholinesterase (AChE) inhibition, neuroprotection, and neurite outgrowth assays in two different neuronal cell-lines (SHS-Y5Y and PC-12 Cells). The test formulation divided into two parts; one part was received the Biofield Energy Treatment by Mr. Mahendra Kumar Trivedi and termed as the Biofield Energy Treated sample, while the other part was denoted as the untreated test sample. Cell viability using MTT assay in SHS-Y5Y and PC-12 cells showed more than 83% cells were viable upto 10.41 µg/mL of the tested concentrations, indicating that the test formulation was safe and nontoxic. The inhibition of AChE enzyme activity was observed by 2.9% in the Biofield Energy Treated test formulation group at 5 µg/mL compared to the untreated test formulation group. The Biofield Energy Treated test formulation group exhibited by 54.83%, 61.37%, 21.51%, and 20.48% cell viability (in terms of restoration of neural protection) at the concentrations of 0.007, 0.013, 0.052, and 0.104 µg/mL, respectively compared to the MPP^+ group. Moreover, the Biofield Energy Treated test formulation showed 48.07% and 36.54% inhibition of Catechol-O-methyl transferase (COMT) enzyme activity at 0.03 and 0.05 µg/mL, respectively compared to the MPP^+ group. The Biofield Energy Treated test formulation also exhibited 166.67%, 250%, 250%, and 20% increased numbers of differentiating neurite cells at 0.03, 0.05, 0.1, and 1.04 µg/mL, respectively compared to the untreated test formulation group. In summary, the Biofield Energy Treated test formulation significantly inhibited AChE and COMT enzymes activities which can improve the memory and cognition function by increasing cell to cell communication, neuronal protection, and neuronal outgrowth. These data indicated that the Biofield Energy Treated novel proprietary formulation has the potential beneficial effects for Neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, that affect millions of people around the world.

Key words: Nanocurcumin; Cognition Enhancer; MTT; Acetylcholinesterase Activity; Neuronal Protection; COMT

Introduction

Acetylcholine (ACh) is one of the neurotransmitter formed from choline and acetyl coenzyme in the nervous system, responsible for learning and memory performance [1,2]. The central nervous system (CNS) related disorders like Alzheimer's disease (AD) occur due to degradation of ACh by acetylcholinesterase enzyme (AChE). Apart from AChE, oxidative stress also influenced neuronal cell death that further aggravates the neurodegenerative diseases like AD [3]. The catechol-O-methyl transferase (COMT) enzyme that degrades catecholamine like dopamine. From literature, it is anticipated that an increased activity of the dopamine regulating enzyme COMT leads to reduce cognitive performance and simultaneously increased the chances to develop various types of psychiatric disorders [4]. Neurite outgrowth assay is used to determine the effects of a particular substrate or exogenous factor on neuron behavior [5]. The basic characteristics features of neurite formation and maturation are essential for the interconnection of neuronal cell bodies. Neurites formation are directly correlated to the neuropathological disorders, neuronal injury/regeneration, and neuropharmacology research and screening [6]. The Complementary and Alternative Medicine (CAM) has increased in worldwide for the ailment of various disorders [7]. The herbal remedies are the most prevalent therapies due to lack of adverse effects and low cost [8]. The newly formulated test formulation, which was a combination of nanocurcumin along with minerals such as sodium selenate, zinc chloride, and magnesium gluconate. Each ingredient of this formulation has been commonly used as nutraceutical supplement [9-12].

Various scientific data reported the fruitful impact of the Biofield Energy Healing Treatment. For example, restoration of immune function in cervical cancer patients after therapeutic touch, massage therapy in enhancing immune system, etc [13,14].

The National Center for Complementary/Alternative Medicine (NCCAM) has demonstrated and given priority to the energy therapies, as it works by manipulating the energy fields that theoretically surround and penetrate the body [15]. Besides, The National Center of Complementary and Integrative Health (NCCIH) has also recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as Qi Gong, natural products, deep breathing, chiropractic/osteopathic manipulation, yoga, Tai Chi, meditation, massage, homeopathy, special diets, progressive relaxation, acupressure, guided imagery, acupuncture, relaxation techniques, healing touch, hypnotherapy, movement therapy, rolfing structural integration, pilates, mindfulness, Ayurvedic medicine, naturopathy, traditional Chinese herbs and medicines, essential oils, Reiki, aromatherapy, cranial sacral therapy and applied prayer (as is common in all religions, like Christianity, Hinduism, Buddhism and Judaism). Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [16].

CAM therapies have been extensively practiced in the universe with different health perspective [17]. The form of subtle energy can be harnessed and transmitted by individuals into non-living and matters via the process of unique Biofield Energy Transmission process. The Trivedi Effect® has been published in various scientific journals with significant outcomes in many scientific fields such as cancer research, microbiology, genetics, pharmaceutical science, agricultural science, and materials science [18-35]. Considering the above facts and information, authors conducted to evaluate the impact of the Biofield Energy Treated novel proprietary on the test herbomineral formulation for cognition enhancing action with respect to neuronal protection, acetylcholine esterase (AChE) inhibition, COMT inhibition, and neurite outgrowth assays.

Material and Methods

Chemicals and Reagents

Antibiotics solution (penicillin-streptomycin) was procured from Himedia. Ham's F12K and RPMI were purchased from NCCS, Pune, India. Iron sulfate, copper chloride, cholecalciferol (vitamin D_3), sodium carboxymethyl cellulose and nerve growth factor (NGF) were obtained from Sigma Chemical Co. (St. Louis, MO). Curcumin and nanocurcumin were purchased from Sanat Products Ltd., India. Zinc chloride and magnesium (II) gluconate hydrate were obtained from TCI, Japan. Sodium selenate and ascorbic acid (vitamin C) were procured from Alfa Aesar, USA. Tetrahydrocurcumin (THC) was procured from Novel Nutrients, India. Galantamine and selegiline were procured from Clearsynth, India. All the other chemicals used in this experiment were analytical grade procured from India.

Biofield Energy Healing Approach

The test formulation was a combination of eight ingredients *viz*. zinc chloride, ferrous sulfate, sodium selenate, nanocurcumin, copper chloride, magnesium gluconate, vitamin C (ascorbic acid) and vitamin D_3 (cholecalciferol). Each ingredient of the test formulation was divided into two parts. One part of each ingredient was considered as control, where no Biofield Energy Healing Treatment was provided to these ingredients. Further, the control groups were treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Healing Treatment. Second part of each ingredients received Biofield Energy Healing Treatment (known as The Trivedi Effect®) under laboratory conditions for 3 minutes through the Healer's unique Energy Transmission process to the test formulation. Biofield Energy Healer, Mahendra Kumar Trivedi in this study did not visit the laboratory, nor had any contact with the herbomineral samples. After that, the Biofield Energy Treated and untreated ingredients were kept in similar sealed conditions and used for the study as per the study plan.

MTT Assay

Cytotoxicity was determined by exposing cells (SHS-Y5Y and PC12) to different concentrations of test formulation in Ham's F12K and RPMI growth medium, respectively. The respective vehicle control kept in the assay was DMSO with LPS. The single cell suspension of SHS-Y5Y in Ham's F12K medium containing 10% FBS was plated at a density of 1x104 cells/well/180µL in 96-well culture plates. Cells were treated with the untreated and Biofield Energy Treated test formulation at concentrations ranging from 0.003 µg/mL to 10.41 µg/mL. After treatment, cells were incubated in a CO_2 incubator at 37 °C and 5% CO_2 for 72 hours. Effect of the test formulation on the viability of SHS-Y5Y was estimated by 3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. 20 µL of 5 mg/mL of MTT was added to all the wells and incubated at 37 °C for 3 hours. Cells were centrifuged and supernatant was removed. The cell pellet in each well was resuspended in 150 µL of DMSO to dissolve formazan crystals. The O.D. of each well was read at 540 nm using Biotek Reader. The number of viable cells was estimated based on the conversion of MTT to formazan dye using a mitochondrial enzyme. The effect of the test formulation on cell viability of splenocytes was determined with the help of the following equation 1:

$$\%\,Cell\ viability = \left(100 - \%\,cytotoxicity\right) \quad (1)$$

Where; % Cytotoxicity = {(O.D. of Control cells – O.D. of cells treated with test formulation)/ OD of Control cells}*100 [36].

Acetylcholinesterase (AChE) Inhibition

AChE activity was measured using a 96-well microplate assay based on Ellman's method [37]. AChE (0.25 to 0.5 U/mL), DTNB (3 mM), and positive control/ test formulation at the different concentrations to be evaluated were mixed and incubated for 15 minutes.

Absorbance was measured at 412 nm. These readings were used as blank readings. The enzymatic reaction was initiated by adding acetylthiocholine iodide (15 mM) and absorbance was measured after 5 min using Synergy HT micro plate reader. Percentage inhibition was calculated using Equation 2.

$$\%Inhibition = \left(1 - \frac{S}{E}\right) * 100 \quad (2)$$

Where, E: Absorbance pertaining reaction mixture (enzyme + substrate)
S: Absorbance pertaining reaction mixture (enzyme + substrate + test formulation/positive control)

Assay for Neuroprotection against MPP^+ and COMT Inhibition

SHS-Y5Y cells were counted using hemocytometer and plated in 96-well plates at the density corresponding to 1 X 10^4 cells/ well followed by overnight incubation in a CO_2 incubator at 37 °C, 5% CO_2, and 95% humidity. Following overnight incubation, the cells were treated with the untreated and Biofield Energy Treated test formulation and positive control at non-cytotoxic concentrations. The cells corresponding to positive control group were treated with selegiline, THC, and curcumin. After 24 hours of pre-treatment with the test formulation or positive control, the cells were treated with MPP^+ (600 μM) for additional 48 hours to induce neuronal damage [38]. Cells were treated with MPP^+ alone served as negative control.

Neuroprotection Assay: After incubation, the plates were taken out and MTT assay was performed as per standard in-house protocol. The percentage of cell viability corresponding to each treatment was calculated using formula 3

$$\%Cell\ viability = 100 - \left[\left(1 - \frac{X}{R}\right) * 100\right] \quad (3)$$

Where, X = OD of wells corresponding to treated cells
R = OD of cells treated with MPP^+

COMT Assay

After incubation, cell lysates were prepared by freeze-thaw method and COMT inhibition was assessed using human catechol-O-methyltransferase (COMT) ELISA kit (Catalog # K12-5709) [39]. The percentage COMT inhibition corresponding to treatment was calculated using Equation 4.

$$\%COMT\ Inhibition = 100 - \left[\left(1 - \frac{X}{R}\right) * 100\right] \quad (4)$$

Where, X = OD of wells corresponding to treated cells
R = OD of untreated cells (Cells maintained in growth medium only)

Neurite Outgrowth Assay

PC-12 cells were trypsinized, counted and plated in collagen coated 24-well plate at the density corresponding to 2x10^4 cells/ well/900μL of growth medium. After overnight incubation cells were serum starved for 3 hours in the medium containing 1% horse serum and 0.5% FBS. The cells were then treated with positive control/test formulation (untreated/Biofield Energy Treated) and incubated in CO2 incubator at 37 °C, 5% CO_2 and 95% humidity. After 48 hours, imaging was done and the number of neurites in differentiating cells was counted. Neurites were defined as extensions from the cell surface that varied in number from 1-5/ cell. The observations were photo-documented using phase contrast microscope in three different fields (magnification: 200x) [40,41].

Fold increase in number of neurites was calculated by Equation 5.

$$Fold\ increase = Number\ of\ neurites\ in\ treated\ \frac{cells}{number}\ of\ neurites\ in\ untreated\ cell \quad (5)$$

Statistical Analysis

Data analysis was performed with Sigma Plot Statistical Software (Version 11.0). Differences between means were assessed for

statistical differences using one-way analysis of variance (ANOVA). $P < 0.05$ was statistically significant. The results are shown as the mean ± standard error of mean (SEM).

Results and Discussion

Cell Viability by MTT Assay in SHS-Y5Y Cells

The effect of the test formulation on cell viability is shown in Figure 1. The untreated cells group showed 100% cell viability. The positive control, selegiline showed more than 74% cell viability and considered as safe upto 10 µM concentrations. Besides, the untreated test formulation and Biofield Energy Treated test formulation showed more than 83% and 88% cell viability, respectively upto 10.41 µg/mL. Overall, the test formulation was found as safe upto 10.41 µg/mL. The percentage cell viability was increased in some of the tested concentrations with respect to the untreated cells, which might be due to the proliferation in cell culture (Figure 1).

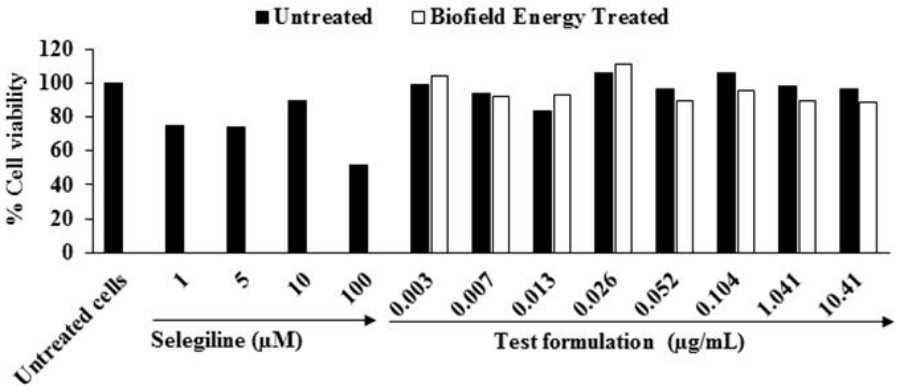


Figure 1: Measurement of Cell Viability Expressed as Percentage by MTT Assay in SHS-Y5Y Cells

Cell Viability by MTT in PC-12 Cells

The cell viability of the test formulation on PC-12 cells is shown in Figure 2. The untreated cells group showed 100% cell viability. The positive control nerve growth factor (NGF) showed a concentration-dependent trend of cell viability of more than 77% upto 500 ng/mL. However, positive control, selegiline showed more than 93% cell viability upto 100 µM concentration. Besides, the untreated test formulation and Biofield Energy Treated test formulation showed more than 94% and 109% cell viability, respectively upto 10 µg/mL. Overall, the test formulation was found as safe upto 10 µg/mL in PC-12 cells. At four concentrations, the level of cell viability was increased in the Biofield Energy Treated test formulation group compared to the untreated test formulation group. The percentage of cell viability was increased in some of the tested concentrations with respect to the untreated cells group, which might be due to the proliferation in cell culture (Figure 2).

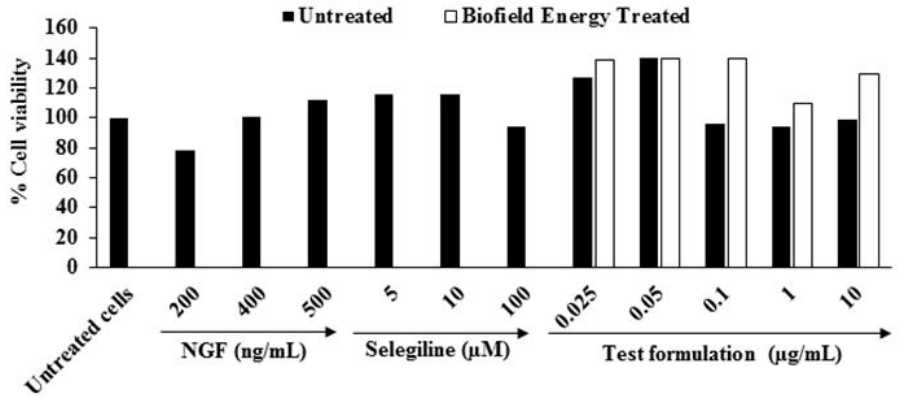


Figure 2: Measurement of Cell Viability by MTT assay in PC-12 cells. Values are represented as Percentage.
NGF; Nerve Growth Factor

Acetylcholinesterase (AChE) Inhibition

Acetylcholinesterase (AChE) is responsible for the termination of impulse transmission through hydrolysis of the neurotransmitter acetylcholine (Ach) in numerous cholinergic pathways in the central and peripheral nervous systems. Inactivation of this enzyme leads to Ach accumulation, hyper-stimulation of nicotinic (N) and muscarinic (M) receptors, and disrupted neurotransmission. Hence, AChE inhibitors both reversible and irreversibly applied in neurodegenerative disorders treatment [42,43]. The level of

AChE activity in the normal control (NC) and vehicle control (VC) group was 100% and 89.1%, respectively. Further, the positive control, galantamine showed a concentration-dependent inhibition of AChE enzyme activity by 28.06%, 35.35%, 68.57%, and 77.55%, respectively compared to the VC group (Figure 3). At 5 µg/mL, the Biofield Energy Treated test formulation showed more inhibition (2.9%) of AChE activity compared to the untreated test formulation group.

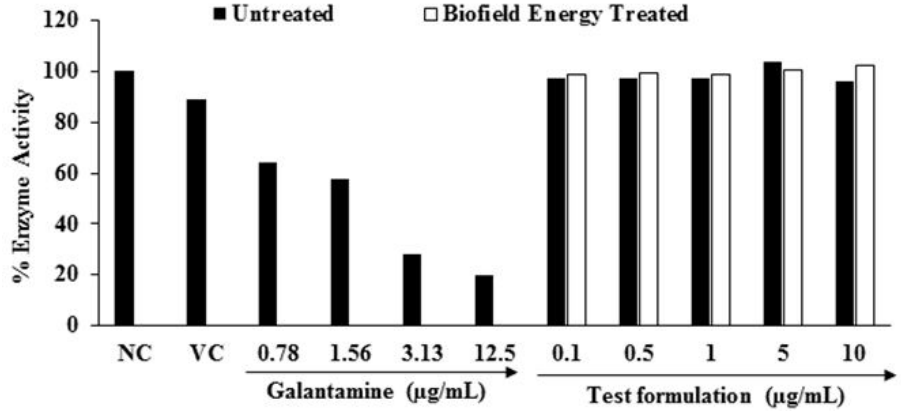


Figure 3: Effect of the test formulation on acetylcholine esterase (AChE) enzyme activity. NC: Normal control; VC: Vehicle control

Assay for Neuroprotection against MPP+ and COMT Inhibition

Neuroprotection Assay: The effect of test formulation on the protection of nerve response or the loss of cell viability after exposure with neurotoxin MPP+ in SHS-Y5Y cells is represented in Figure 4. The cell viability in the untreated cell group was 100%, while it was significantly reduced by 71.73% in the MPP+ group. Moreover, the positive control, selegiline was significantly increased/or restored cell viability in a concentration-dependent manner by 18.75%, 18.85%, 37.64%, and 50.87%, at the concentration of 1, 5, 10, and 100 µM, respectively compared to the MPP+ group. The untreated test formulation was significantly increased the cell viability by 67.21%, 34.14%, 12.38%, 19.49%, 26.46%, and 7.7% at the concentrations of 0.003, 0.007, 0.013, 0.026, 0.052, and 0.104 µg/mL, respectively compared to the MPP+ group. However, the Biofield Energy Treated test formulation group exhibited by 54.83%, 61.37%, 11.32%, 21.51%, 20.48%, and 10.05% cell viability at the concentrations of 0.007, 0.013, 0.026, 0.052, 0.104, and 1.041 µg/mL, respectively compared to the MPP+ group (Figure 4). Overall, at four concentrations viz. 0.007, 0.013, 0.104, and 1.041 µg/mL the Biofield Energy Treated test formulation showed improved cell viability by 15.18%, 42.56%, 15.53%, and 11.11%, respectively compared to the untreated test formulation group.

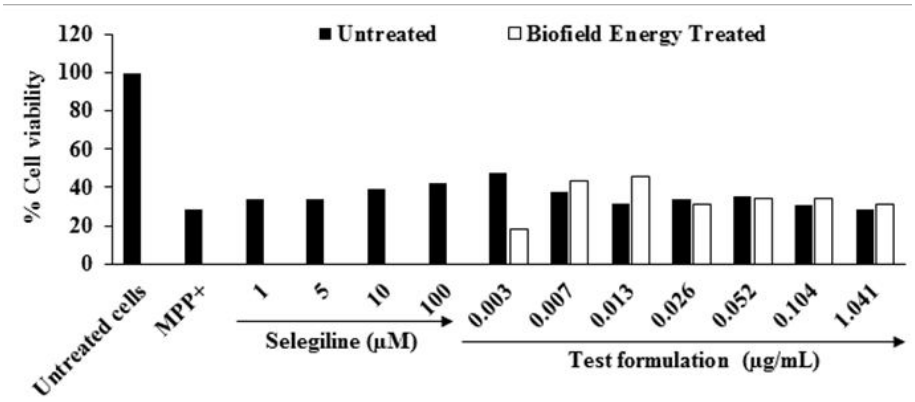


Figure 4: Effect of the test formulation on neuronal protection activity against MPP+ induction. MPP+: N-methyl-4-phenylpyridinium

COMT Assay

The COMT protein can exists in the two forms a) the soluble form (S-COMT), which predominates in the peripheral nervous system (PNS), and b) the membrane-bound form (MB-COMT), which is more abundant in the brain [44]. The wide spectrum effects of COMT on behavior are due to the widespread expression of this enzyme in the brain and its vital role in the metabolism of catecholamine neurotransmitters [45]. The level of COMT enzyme concentration in the untreated cells group was 1.79 µM. While, it was increased by 16.20% in the MPP+ group. Further, the positive control selegiline significantly inhibited the COMT enzyme activity by 10.58%, 3.37%, and 24.04% at the concentrations of 0.1, 1, and 10 µM, respectively compared to the MPP+ group. The untreated test formulation showed 24.52%, 25%, 35.58%, 31.73%, and 30.29% inhibition of COMT enzyme function at 0.03, 0.05, 0.10, 1.04, and 10.41 µg/mL, respectively compared to the MPP+ group. However, Biofield Energy Treated test formulation

showed 48.07%, 36.54%, 17.31%, and 10.58% inhibition of COMT enzyme activity at 0.03, 0.05, 0.10, and 1.04 µg/mL, respectively compared to the MPP^+ group (Figure 5). Overall, the Biofield Energy Treated test formulation showed more inhibition of COMT enzyme activity at 0.03 and 0.05 µg/mL by 31.21% and 15.38%, respectively compared to untreated test formulation group.

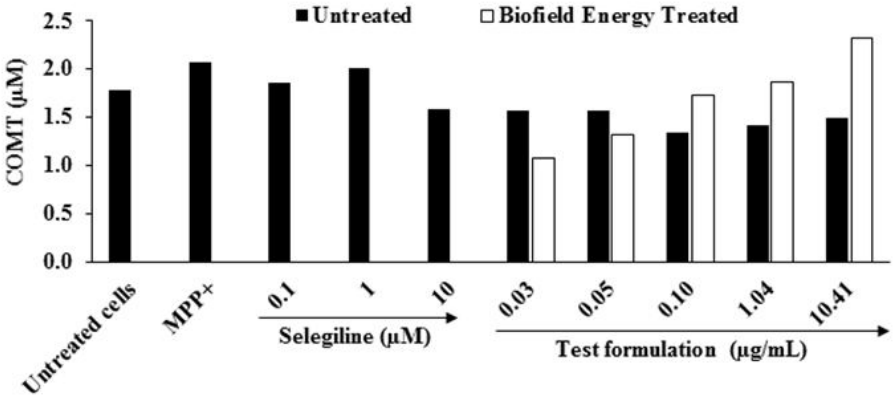


Figure 5: Effect of the Test Formulation on COMT Enzyme Activity against MPP^+ induction. MPP^+: N-methyl-4-phenylpyridinium

Neurite Outgrowth Assay

Neurite outgrowth assay is used to phenotypic screen the effects of the test formulation on neuronal cells. In this experiment, a manual analytical method was performed with the help of photo-micrographic images to measure the neurite formation, elongation, and regression on differentiated and undifferentiated neurons (Figure 7). Neurites are very delicate organelles and require attachment to membranes for both signaling and recovery, which involve for the detection of macromolecular signals by the growth cone, a complex sensing apparatus that caps the neurite extension [46-48]. The number of neurite cells in the untreated cells was 6. The positive control nerve growth factor (NGF) showed the number of differentiating neurite cells as 155, 186, and 175 at the concentration of 200, 400, and 500 ng/mL, respectively. Moreover, other positive control group like selegiline showed the number of differentiating neurite cells as 7 and 33 at the concentration of 5 and 10 µM, respectively. The untreated test formulation showed 50% and 44.44% increased the numbers of differentiating neurite cells at 0.03 and 10.41 µg/mL, respectively compared to the untreated cells group. Besides, the Biofield Energy Treated test formulation showed 300%, 250%, 133.33%, and 66.67% increased the numbers of differentiating neurite cells at 0.03, 0.05, 0.10, and 10.41 µg/mL, respectively than untreated (Figure 6). Overall, the Biofield Energy Treated test formulation exhibited 166.67%, 250%, 250%, and 20% increased the numbers of differentiating neurite cells at 0.03, 0.05, 0.1, and 1.04 µg/mL, respectively compared to the untreated test formulation group.

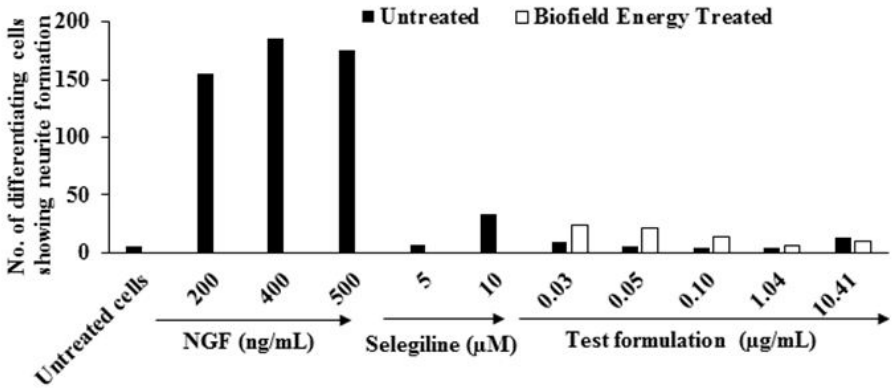
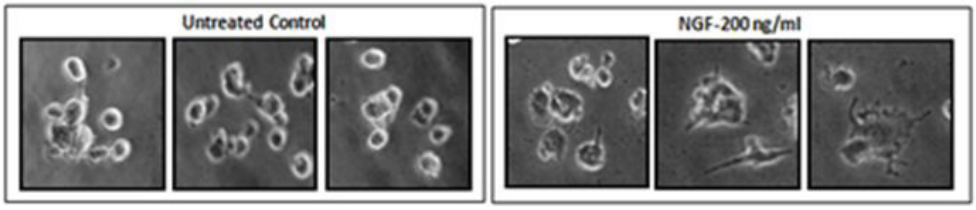


Figure 6: Effect of the Test Formulation on Neurite Outgrowth after NGF Induction. NGF: Nerve Growth Factor



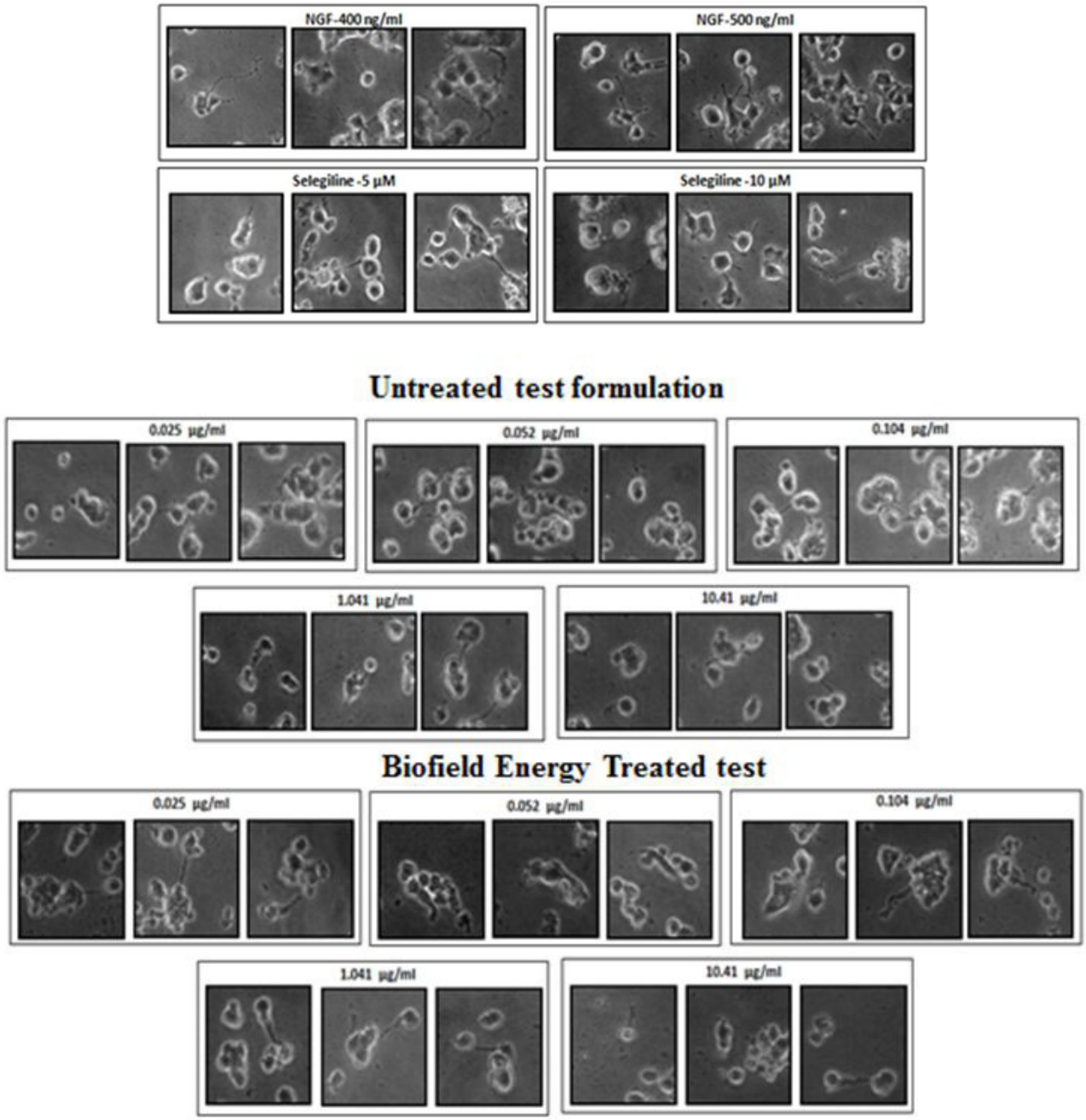


Figure 7: Representative Images of Neurite outgrowth after Exposure with the Test Formulation

Conclusion

The cell viability was assessed using MTT assay in SHS-Y5Y and PC-12 cells showed more than 83% cells were viable upto 10.41 µg/mL of the tested concentrations, indicating that the test formulation was safe and nontoxic. The Biofield Energy Treated test formulation showed 2.9% inhibition of acetylcholinesterase (AChE) enzyme activity at 5 µg/mL compared to the untreated test formulation group. Additionally, 54.83%, 61.37%, 21.51%, and 20.48% neuronal cells protection was observed in the Biofield Energy Treated test formulation group at 0.007, 0.013, 0.052, and 0.104 µg/mL, respectively compared to the MPP^+ group. Moreover, the COMT enzyme activity was significantly inhibited by 48.07% and 36.54% at 0.03 and 0.05 µg/mL, respectively compared to the MPP^+ group. The Biofield Energy Treated test formulation exhibited 166.67%, 250%, 250%, and 20% increased the numbers of differentiating neurite cells at 0.03, 0.05, 0.1, and 1.04 µg/mL, respectively compared to the untreated test formulation group. The study results summarized that the Biofield Energy Treated test formulation significantly inhibited AChE and COMT enzymes activities which can improve the memory and cognition function by increasing cell to cell communication, neuronal protection, and neuronal outgrowth. Novel proprietary test formulation showed significant modulation of cognition activity compared to the untreated test formulation group in different cell lines. Overall, data indicated that the memory and cognition power can be improved after the exposure with of the Biofield Energy Treated novel proprietary formulation. It is then anticipated that the Biofield Energy Treated formulation could be more useful to enhance memory and cognitive functions for healthy human and

in patients who suffer with the neurological disorders. These data indicated that the Biofield Energy Treated novel proprietary formulation has the potential beneficial effects for Neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Its uses can be extendable for organ transplant like kidney, liver and heart, and numerous autoimmune disorders like Multiple Sclerosis, Myasthenia Gravis, Rheumatoid Arthritis, Aplastic Anemia, Pernicious Anemia, etc. Apart from these, the Biofield Treated test formulation could be used against various inflammatory disorders like Irritable Bowel Syndrome, Ulcerative Colitis, Dermatitis, and stress etc. to modulate the immune system by improving overall health.

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