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Investigation of Biofield Energy Treated Proprietary Test Formulation for Cognition Biomarkers in Brain and CSF Using Unpredictable Chronic Stress (UCS) Animal Model

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Abstract

Cognition biomarkers in brain (GABA, Glutamate, Beta Endorphin) and CSF (Corticosterone, KLOTHO, and Serotonin) were evaluated using unpredictable chronic stress (UCS) rodent model in presence of Consciousness Energy Healing Treated (known as the Trivedi Effect®) novel test formulation in male Sprague Dawley (SD) rats using ELISA assay. The test formulation was consisted of minerals (Zn, Fe, Cu, Se, Ca, Mg), vitamins (C, E, B6, B12, D3), β-carotene, ginseng, and cannabidiol isolate. The test formulation constituents were divided into two parts, one part of each ingredient was distinct as the untreated test formulation, while the other portion of the test formulation and a group of animals received Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The level of brain GABA in groups viz. G7 (15-days pre-treatment of the Biofield Energy Treated Test formulation) and G8 (15-days pre-treatment of the Biofield Energy Treated Test formulation to the Biofield Energy Treatment *per se* rats) was increased by 12.6% and 14.3%, respectively as compared with the disease control group (G2). Brain glutamate was increased by 20.6%, 36.5%, 25.3%, and 40.7% in the G6 (Biofield Energy Treatment *per se* to the rats), G7, G8, and G9 (untreated test formulation to the Biofield Energy Treatment *per se* to the rats) groups respectively, as compared with the G2. Brain beta endorphin was significantly increased by 114.3% and 112% in the G5 (Biofield Energy Treated Test formulation to the untreated rats) and G6 groups respectively, as compared with the G4. Similarly, the level of corticosterone in CSF was significantly decreased by 25%, 28.7%, 25%, 27.7%, and 26.9% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. The level of KLOTHO in CSF was significantly increased by 17.6%, 83.3%, 36.4%, and 102.8% in the G5, G6, G7, and G8 groups respectively, as compared with the G4. These data suggested that the Biofield Energy *per se* significantly reduce the stress-related biomarker such as corticosterone and significantly improve cognition related biomarkers such as GABA, Glutamate, Beta Endorphin, and KLOTHO protein. The brain neurotransmitters (GABA, and glutamate) level was significantly increased in the different treatment groups as compared to the disease control group, which played a vital role in animal's cognitive functions. Overall, the results showed the significant slowdown the stress-related disease progression and its complications/symptoms in rats in the preventive Biofield Energy Treatment group *per se* and/or Biofield Energy Treated Test formulation groups (*viz*. G6, G7, G8, and G9) comparatively with the disease group.

Keywords: Biofield Treatment; GABA; Glutamate; Beta Endorphin; Corticosterone; KLOTHO; Serotonin; The Trivedi Effect®; Unpredictable Chronic Stress; ELISA

Abbreviations: UCS: Unpredictable Chronic Stress; PNS: Peripheral Nervous System; SD: Sprague Dawley; HPA: Hypothalamus-pituitary-adrenal; GABA: Gamma Aminobutyric Acid; CNS: Central Nervous System; CAM: Complementary and Alternative Medicine; NCCAM: National Center for Complementary Alternative Medicine; TIA:

Transient Ischemic Attack.

Introduction

Stress includes physiological responses at the central (arousal, vigilance, and attention) and peripheral systems (metabolism and oxidation) to minimize the effects of that stressor [1-3]. Body stressors mainly act on the autonomic nervous system in order to prepare for any upcoming classic "fight or flight" response, which protect from any immediate danger [4-7]. Hypothalamus-pituitary-adrenal (HPA) axis is also engaged in chronic stress conditions to minimize the neuroendocrine response kinetics, which may dangerous to the body organs [8]. Over thousands of studies confirmed the beneficial role of major critical brain and CSF chemical mediators, which regulates and balanced the deleterious effects of chronic stress. Brain GABA (gamma aminobutyric acid) also known as calm chemical, one of the major inhibitory neurotransmitters in central nervous system. Brain endorphins are a category of neurotransmitters, which works as an internal pain killer acts as feel-good effect. Serotonin is regarded as the happy neurotransmitters, which relay signals from one part of the brain to another. It greatly impacts on mood, contributing greatly to our overall state of well-being. GABA, glutamate, and serotonin are the most plentiful neurotransmitters found in the central nervous system (CNS) where they play a role in cognitive functions such as memory and learning [9-12]. There are various pharmaceutical/nutraceutical/dietary supplement formulations that have been developed by the scientific community to solve the complications arising due to Unpredictable Chronic Stress (UCS), either by rectifying the root cause or improving the associated symptoms [13], that can regulate the level of cognition biomarkers in brain (GABA, Glutamate, Beta Endorphin) and CSF (Corticosterone, KLOTHO, Serotonin).

Thus, in order to maintain the level of cognition biomarkers in presence of unpredictable stress, a novel test formulation was designed with the combination of vital minerals (selenium, zinc, iron, calcium, copper, and magnesium), essential vitamins (cyanocobalamin, ascorbic acid, pyridoxine HCl, alpha tocopherol, and cholecalciferol), β-carotene, Ginseng, and cannabidiol isolate (CBD)). All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological role [14-18], while ginseng extract is regarded as the one of the best immune booster for overall immunity [19]. The present study aimed to evaluate the cognition biomarkers in brain and CSF of Sprague Dawley rats in unpredictable chronic stress (UCS), which was treated with Biofield Energy Treatment (a Complementary and Alternative Medicine, CAM) by a renowned Biofield Energy Healer. Biofield Energy Healing Treatment, one of the emerging CAM approaches with significant results that has the capacity to improve overall immunity and anti-inflammatory activity. Biofield Energy Healing is a novel approach that has been known widely used against various pathological conditions [20,21]. CAM therapies are considered as the one among best alternative complementary health treatment approach by the National Center for Complementary/ Alternative Medicine (NCCAM) [22], due to its several benefits over the current preferred treatment approach [23]. Various other CAM therapies, medicines and practices has been reported worldwide such as Tai Chi, deep breathing, yoga, natural products, therapeutic touch, Qi Gong, Reiki, pranic healing, polarity therapy, chiropractic manipulation, special diets, meditation, homeopathy, mindfulness, progressive relaxation, movement therapy, pilates, Ayurvedic medicine, and traditional Chinese herbs and medicines in biological systems, etc [24,25]. Similarly, the Trivedi Effect®-Consciousness Energy Healing therapy has been known worldwide as a Conventional therapy due to its significant impact in various living and non-living objects. The impact of the Trivedi Effect® has been scientifically studied in various scientific fields such as, agriculture science [26], materials science [27,28], microbiology [29,30], biotechnology [31,32], skin health [33,34], bioavailability studies [35,36], nutraceuticals [37], bone health [38-40], cancer research [41], and overall human health and wellness. In this study, the authors evaluated the impact of the Biofield Energy Treatment (the Trivedi Effect®) for the level of cognition biomarkers in brain (GABA, glutamate, and beta Endorphin) and CSF (Corticosterone, KLOTHO, and serotonin) in Sprague Dawley rats using elisa assays.

Material and Methods

Chemicals and reagents

The novel proprietary test formulation was constituted with pyridoxine hydrochloride (vitamin B_6), calcitriol, zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, Provit A), which were purchased from TCI, Japan. Copper chloride, cyanocobalamin (vitamin B_{12}), calcium chloride, vitamin E (alpha-tocopherol), cholecalciferol (vitamin D_3), iron (II) sulfate, and sodium carboxymethyl cellulose (Na-CMC) were procured from Sigma-Aldrich, USA. Ascorbic acid (vitamin C) and sodium selenate were obtained from Alfa Aesar, India. Cannabidiol isolate and Panax ginseng extract were obtained from Panacea Phytoextracts, India and Standard Hemp Company, USA, respectively. Imipramine Hydrochloride was purchased from Sigma, USA. For the estimation of cognition biomarker in brain (GABA, Glutamate, Beta Endorphin) and CSF (Corticosterone, KLOTHO, Serotonin) using ELISA kits, which were used for the detection. GABA and Glutamate was procured from Qayee-Bio and My Bio Source, USA respectively. However, Beta Endorphin, corticosterone, KLOTHO, and serotonin were procured from CUSABIO, USA.

Maintenance of animal

Randomly breed male Sprague Dawley (SD) rats with body weight ranges from 200 to 300gm were used in this study. The animals were purchased from M/s. Vivo Bio Tech, Hyderabad, India. Animals were randomly divided into nine groups based on their body weights consist of 6 animals of each group. They were kept individually in sterilized polypropylene cages with stainless steel top grill having provision for holding pellet feed and drinking water bottle fitted with stainless steel sipper tube. The animals were maintained as per standard protocol throughout the experiment.

Consciousness energy healing strategies

Consciousness Energy healing treatment was designed by dividing each constituents of the test formulation into two parts. One part each of the test formulation constituents did not received any sort of treatment and was labeled as the untreated or control sample, while second part of the test formulation was treated with the Trivedi Effect® - Energy of Consciousness Healing Treatment (Biofield Energy Treatment) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under standard laboratory conditions for ~3 minutes. The novel test formulation was consisted of zinc chloride, iron (II) sulfate, copper chloride, vitamin B_6, vitamin B_{12}, vitamin D_3, sodium selenate, calcium chloride, ascorbic acid, vitamin E, beta carotene, *Panax ginseng* extract, cannabidiol, and magnesium (II) gluconate. Besides, three group of animals (n=10/per group) also received Biofield Energy Healing Treatment (known as the Trivedi Effect®) by Mr. Mahendra Kumar Trivedi under similar laboratory conditions for ~3 minutes. The Biofield Energy Healer was located in the USA, however the test formulation were located in the research laboratory of Dabur Research Foundation, New Delhi, India. The energy transmission was done without touching the samples or animals. After that, the Biofield Energy Treated samples was kept in the similar sealed condition and used as per the study plan. In the same manner, the control test formulation group was subjected to "sham" healer under the same laboratory conditions. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. The Biofield Energy Treated animals were also taken back to experimental room for further proceedings.

Experimental test procedure

Seven days after acclimatization, animals were randomized and grouped based on the body weight. The test formulation was prepared freshly prior to dosing and administered to the animals using an oral intubation needle attached to an appropriately graduated disposable syringe. The dose volume was 10 mL/kg in morning and evening based on body weight. The experimental groups were divided as G1 as normal control; G2 as disease control (UCS: Unpredictable Chronic Stress with 0.5% CMC); G3 as reference item (UCS along with imipramine hydrochloride, 30 mg/kg); G4 includes UCS along with untreated test formulation; G5 as UCS along with Biofield Energy Treated test formulation); G6 group includes UCS along with Biofield Energy Treatment *per se* to animals from day -15; G7 as UCS along with Biofield Energy Treated test formulation from day -15; G8 group includes UCS along with Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9 group denoted UCS along with Biofield Energy Treatment *per se* animals plus untreated test formulation. G1 and G2 animals were treated orally with 0.5% w/v CMC-Na in distilled water for 8 weeks (From day 1 to 56). Group G3 animal was treated orally with reference item, imipramine hydrochloride at a dose of 30 mg/kg body weight for 8 weeks. The freshly prepared suspensions of untreated test formulation and Biofield Energy Treated Proprietary Product was administered orally to the G4 and G5 group animals, respectively, at a dose of 990.56 mg/kg BW−1·d−1 for 8 weeks by oral route. G6 group did not dose with the test formulation. In addition; G7 and G8 groups were dosed similar to the G4 and G5 dosing regimen, but from the day of Biofield Energy Treatment (i.e. from day-15 to day 56). G9 group, Biofield Energy Treated *per se* animal was treated with untreated test formulation for 8 weeks. Body weight and clinical signs were taken daily throughout the experimental period. All the animals except G1 group received stress induced procedures such as stress procedures like sound stress, tilted cages and crowd stress, cold and warm water swim stress, food and water deprivation, stress due to change in the light and dark cycle were undergo seven different types of unpredictable stress procedures after scheduled dosing daily at specified interval to the end of the experiment for 8 weeks after the initiation of stress, which vary every week interval i.e., shuffling of stress type. At the end of (8 week) experimental period, all the animals were individually subjected gross necropsy to collect brain tissue for the experimental purpose.

Preparation of Sample for Elisa Assay

With the continued stress treatment of 4th week of the experimental period, all the animals were individually subjected for blood collection using retro-orbital route and the blood was collected in the plain vial, which was used for the separation of serum in all the animals of different experimental groups. The serum from all the groups was stored at -20°C for further estimation. Alternatively, aliquot all the samples and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles, which may alter the level in brain (GABA, Glutamate, Beta Endorphin) and CSF (Corticosterone, KLOTHO, Serotonin) during the final calculations.

Estimation of Brain (GABA, Glutamate, Beta Endorphin) and CSF (Corticosterone, KLOTHO, Serotonin) Biomarkers

The serum from all the animals groups after experimental period was subjected for the estimation of brain (GABA, Glutamate, Beta Endorphin) and CSF (Corticosterone, KLOTHO, Serotonin) biomarkers. The entire assay was estimation using ELISA method as per manufacturer's recommended standard procedure. This was a quantitative method and the principle was based on the binding of protein and their specific antibody.

Statistical Analysis

The data were represented as mean ± standard error of mean (SEM) and subjected to statistical analysis using Sigma-Plot statistical software (Version 11.0). For multiple comparison One-way analysis of variance (ANOVA) followed by post-hoc analysis by Dunnett's test and for between two groups comparison Student's *t*-test was performed. The $p \leq 0.05$ was considered as statistically significant.

Results and Discussion

Estimation of brain gaba

GABA, a naturally occurring amino acid brain neurotransmitter, which attaches to a protein named as GABA receptor responsible for calming effect. Besides, it has been reported as one of the important biomarker for neuropsychiatric diseases, which has significance role in anxiety, stress, and fear. It may also help to prevent seizures [42-44]. Brain GABA level was estimated in all the experimental test group and the results are presented in Figure 1. Brain GABA level in unpredictable chronic stress (G2) was found to be 37.54±0.67 ng/mL, which was decreased as compared to normal control (G1, 36.71±0.45ng/mL) group. Imipramine treatment (G3) increased brain GABA level (39.48±1.10ng/mL) by 5.2% as compared to the G2. The untreated test formulation to the untreated rats (G4) increased brain GABA level (40.49±0.67ng/mL) by 7.9% as compared to the G2. The Biofield Energy Treated test formulation to the untreated rats (G5) was significantly increased brain GABA level (41.20 ± 0.61ng/mL) by 9.8% as compared to the G2. Biofield Energy Treatment per se to the rats (G6) was increased brain GABA level (40.49 ± 0.54 ng/mL) by 7.9% as compared to G2. 15-days pre-treatment of the Biofield Energy Treated test formulation (G7) was significantly increased brain GABA level (42.25±1.15ng/mL) by 12.6% and 4.3% as compared to the G2 and G4, respectively. 15-days pre-treatment of the Biofield Energy Treated test formulation to the Biofield Energy Treated rats (G8) significantly increased brain GABA level (42.90±1.14 ng/mL) by 14.3% and 5.9% as compared to the G2 and G4, respectively. The untreated test formulation to the Biofield Energy Treated rats (G9) significantly increased brain GABA level (40.88 ± 0.93 ng/mL) by 8.9% as compared to the G2.

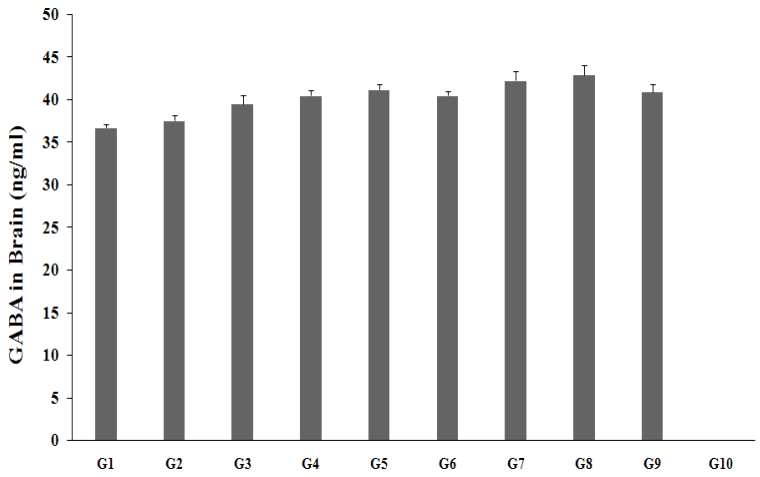

Figure 1: The effect of the test formulation on the level of brain GABA in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Estimation of Brain Glutamate

Brain glutamate is another vital and one of the abundant neurotransmitter in central nervous system (CNS). It has important role in managing the neural circuits involved with synaptic plasticity by strengthening or weakening the signalling process over time to shape up the learning memory. Glutamate in brain is very important as little level can result in difficulty concentrating or mental exhaustion, while high level results in excitotoxicity that can damage nerve cells or neurons [45-47]. Brain glutamate level was estimated in all the experimental test group and the results are presented in Figure 2. Brain glutamate level in unpredictable chronic stress (G2) was 14.41±0.93ng/mL, which was decreased by 12.1% as compared to the normal control (G1, 16.39 ± 1.44ng/mL). Imipramine treatment (G3) increased brain glutamate level (15.67 ± 1.44 ng/mL) by 8.7% as compared to the G2. The untreated test formulation to the untreated rats (G4) showed increased brain glutamate level (16.12 ± 0.68ng/mL) by 11.9% as compared to the G2. G5 group showed an increased brain glutamate level (15.50 ± 1.38ng/mL) by 7.6% as compared to the G2. The level of brain glutamate was increased in the G6 (17.37 ± 0.35 ng/mL), G7 (19.66 ± 1.20 ng/mL), G8 (18.05 ± 1.19 ng/mL), and G9 (20.27± 1.01 ng/mL) by 7.8%, 22%, 12%, and 25.7%, respectively as compared to the G4. Similarly, G6, G7, G8, and G9 group showed an increased brain glutamate by 20.6%, 36.5%, 25.3%, and 40.7%, respectively as compared to the G2.

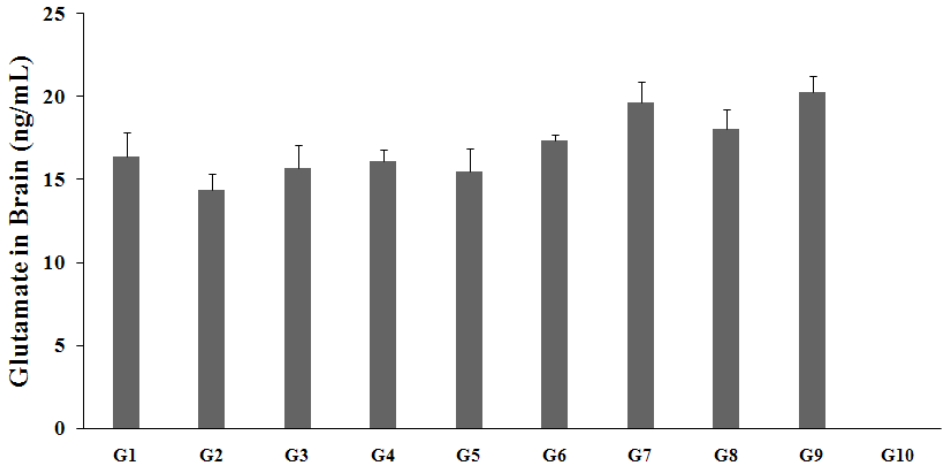

Figure 2: The effect of the test formulation on the level of brain glutamate in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Estimation of Brain Beta Endorphin

Brain beta-endorphin is an opioid neuropeptide that play a vital role in the development of hypotheses concerning the non-synaptic or paracrine communication of brain signals. It plays an important role in peripheral nervous system (PNS), by producing analgesia action after binding with the opioid receptors at synaptic nerve terminals. Thus, endorphins role in physiologic stressors has been widely reported such as pain [48-51]. The present experiment was performed for the estimation of brain glutamate in all the experimental test group and the results are presented in Figure 3. Brain beta endorphin level in unpredictable chronic stress (G2) was 21.01±6.12ng/mL, which was significantly decreased by 87.8% as compared to the normal control (G1, 172.47 ± 80.01ng/mL). Imipramine treatment (G3) significantly increased brain beta endorphin level (43.75 ± 16.26ng/mL) by 108.2% as compared to the G2. Untreated test formulation to the untreated rats (G4) decreased brain beta endorphin (14.82 ± 1.90ng/mL) as compared to the G2. The Biofield Energy Treated test formulation to the untreated rats (G5) was increased brain beta endorphin level (31.74±6.27ng/mL) by 51.1% and 114.3% as compared to the G2 and G4 groups, respectively. G6 group also showed an increased brain beta endorphin level (31.41 ± 9.76 ng/mL) by 49.5% and 112% as compared to the G2 and G4 groups, respectively. G7 group showed decreased brain beta endorphin level (14.73 ± 2.74 ng/mL) as compared to G2. Similarly, G8 (5.20 ± 1.10 ng/mL) and G9 (9.16± 1.91 ng/mL) also showed decreased brain beta endorphin level as compared with G2 group.

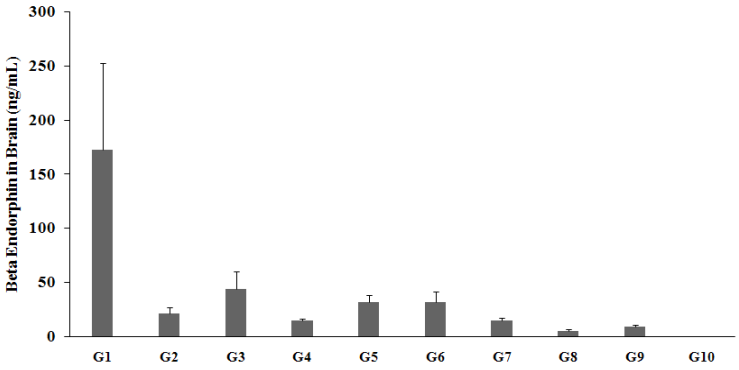

Figure 3: The effect of the test formulation on the level of brain beta endorphin in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Estimation of CSF Corticosterone

Corticosterone, the primary glucocorticoid has wide application in anxiety and depressive along with many severe neuropsychiatric syndromes. Corticosterone dysregulation mainly affects older people with chronic cognitive impairment commonly precipitated by acute illness, surgery, and trauma [52]. The CSF corticosterone estimation was performed in all the experimental test group and the results are presented in Figure 4. CSF corticosterone level in unpredictable chronic stress (G2) was 2.01±0.12ng/mL, which was significantly increased by 24.9% in comparison with normal control (G1, 1.61 ± 0.16 ng/mL). Imipramine treatment (G3) was significantly decreased CSF corticosterone level (1.61 ± 0.09 ng/mL) by 19.8% as compared to the G2. The untreated test formulation to the untreated rats (G4) was decreased CSF corticosterone level (1.73 ± 0.09 ng/mL) by 13.9% as compared to the G2. G5 (1.51 ± 0.07 ng/mL), G6 (1.44 ± 0.09 ng/mL), G7 (1.51 ± 0.02 ng/mL), G8 (1.45 ± 0.06 ng/mL), and G9 (1.47 ± 0.07 ng/mL) groups showed a significant decreased level of CSF corticosterone by 12.9%, 17.2%, 12.9%, 16.1%, and 15.1% respectively, as compared with the G4 group. However, G5, G6, G7, G8, and G9 groups also showed a significant decreased CSF corticosterone level by 25%, 28.7%, 25%, 27.7%, and 26.9% respectively, as compared with the G2 group.

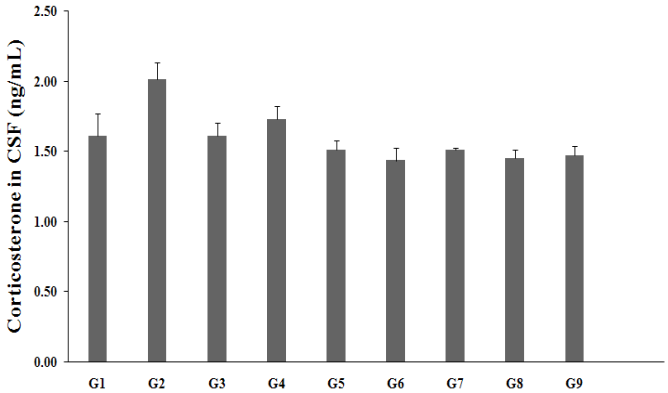

Figure 4: The effect of the test formulation on the level of CSF corticosterone in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment per se to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Estimation of CSF KLOTHO

KLOTHO is a hormone that promotes longevity and regulates the span of life, it also promotes longevity. However, preclinical and clinical data suggested that KLOTHO significantly affects the onset of many premature senescent phenotypes, such as atherosclerosis, cardiovascular disease, stroke and osteoporosis [53,54]. The CSF KLOTHO estimation was performed in all the experimental test group and the results are presented in Figure 5. CSF KLOTHO level in Unpredictable chronic stress (G2) group was 343.29 ± 20.85 pg/mL, which was significantly increased by 51.3% in comparison with the normal control (G1, 705.43 ± 59.49 pg/mL). Imipramine treatment (G3) was increased CSF KLOTHO level (481.86 ± 70.96pg/mL) by 40.4% as compared to the G2. The untreated test formulation to the untreated rats (G4) decreased the CSF KLOTHO level (318.71 ± 19.43pg/mL) as compared to the G2. G5 (374.86 ± 41.77pg/mL), G6 (584.29 ± 87.63 pg/mL), G7 (434.57±67.13pg/mL), G8 (646.29±259.26pg/mL), and G9 (338.14±30.52pg/mL) groups showed significant increased CSF KLOTHO level by 17.6%, 83.3%, 36.4%, 102.8%, and 6.1%, respectively as compared to the G4. However, G5, G6, and G7 groups also showed a significant increased CSF KLOTHO by 9.2%, 70.2%, and 26.6%, respectively as compared to the G2.

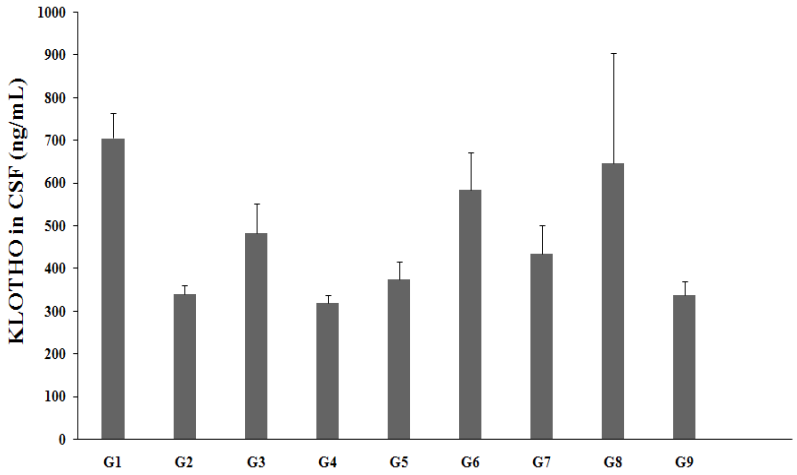

Figure 5: The effect of the test formulation on the level of CSF KLOTHO in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment per se to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Estimation of CSF Serotonin

Serotonin play a vital role and its diminishing activity imparts a crucial role in the pathophysiology of depression and many neurological disorders such as Alzheimer disease, schizophrenia, panic disorder, fibromyalgia, or atypical depression. Thus, the level of serotonin can be maintained by using some alternative way of treatment, which would be the best mode of treatment in neurological disorders [55,56]. The CSF serotonin was estimated in all the experimental test group and the results are presented in Figure 6. CSF Serotonin level in unpredictable chronic stress (G2) was 22.08 ± 2.86 pg/mL, which was decreased by 2.4% as compared with the normal control (G1, 22.62 ± 6.63 pg/mL). Imipramine treatment (G3) significantly decreased the CSF serotonin level (13.57 ± 1.12 pg/mL) by 38.5% as compared to the G2. The untreated test formulation to the untreated rats (G4) decreased the CSF serotonin level (16.63 ± 2.84 pg/mL) by 24.7% as compared to the G2. G5 (14.97 ± 3.86 pg/mL), G6 (12.39 ± 3.81 pg/mL), G7 (10.59 ± 1.94 pg/mL), G8 (12.50 ± 1.10 pg/mL), and G9 (8.43 ± 2.04 pg/mL) groups showed a significant decreased CSF serotonin level by 10%, 25.5%, 36.3%, 24.8%, and 49.3%, respectively as compared with the G4. However, G5, G6, and G7 groups also showed a significant decreased CSF serotonin by 9.2%, 70.2%, and 26.6% respectively, as compared to the G2.

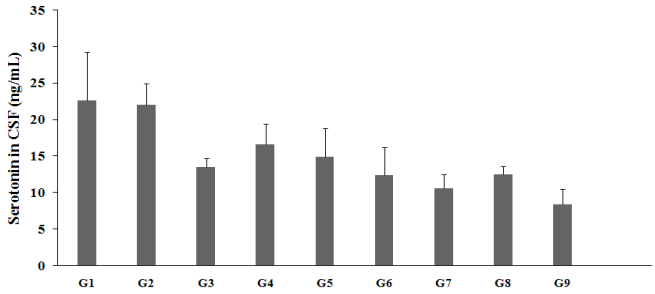

Figure 6**:** The effect of the test formulation on the level of CSF Serotonin in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment per se to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

In this research plan, four groups were considered as preventive maintenance groups. These groups were G6 (Biofield Energy Treatment *per se* to animals at -15 days), G7 (Biofield Energy Treated test formulation from day -15), G8 (Biofield Energy Treatment per se to animals along with Biofield Treated test formulation from day -15), and G9 (Biofield treatment *per se* at -15 days to animals with untreated test formulation). The results showed the significant slowdown of the disease progression, stress disease related all other symptoms/complications and also reduced the chances of disease sUCSeptibility in these groups. Specifically, group G6 (preventive Biofield Energy Treatment group *per se* at -15 days) showed the best results as a prophylactic/preventive treatment group compared to the other groups. Based on the overall data, it suggests that the Biofield Energy Healing Therapy was found to be most effective and benefited in order to prevent and protect from the occurrence of any type of diseases in rat model. It indicated that this therapy can act as a preventive maintenance therapy to prevent the occurrence of the disease, slow down the disease progression and disease related complications of the existing aliments that will ultimately improve the overall health and quality of life in human.

Conclusions

Cognition biomarkers in brain and CSF were identified after treatment with the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* on the animal. The following parameters such as the GABA, Glutamate, Beta Endorphin in Brain, while corticosterone, KLOTHO, and serotonin in CSF were studies in the unpredictable chronic stress (UCS) rodent model. The level of brain GABA was significantly increased by 12.6% and 14.3% in the G7 and G8 groups respectively as compared with the G2. Similarly, brain glutamate was significantly increased by 20.6%, 36.5%, 25.3%, and 40.7% in the G6, G7, G8, and G9 groups respectively, as compared with the G2. Brain beta endorphin was also significantly increased by 114.3% and 112% in the G5 and G6 groups respectively, as compared with the G4. CSF corticosterone level was significantly decreased by 25%, 28.7%, 25%, 27.7%, and 26.9% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2. CSF KLOTHO was significantly increased by 17.6%, 83.3%, 36.4%, and 102.8% in the G5, G6, G7, and G8 groups, respectively as compared with the G4. are the most plentiful The brain neurotransmitters (GABA, and glutamate) level were significantly increased in the different treatment groups as compared to the vehicle control group, which played a vital role in cognitive functions such as memory and learning. Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed best results with respect to different efficacy and biomarker parameters in the preventive maintenance group, G6 as compared to the other preventive maintenance groups (G7, G8, and G9) in rat model study. It also helped to slow down the disease progression and disease-related complications of the overall animal's health. These data suggested that Biofield Energy Treatment *per se* and/or Biofield Energy Treated Test formulation in combination would be the best treatment strategies in order to prevent and protect from the occurrence of any type of diseases. Therefore, The Biofield Energy Treatment might act as a preventive maintenance therapy in order to maintain good health, or full restoration of health or improve the overall health and quality of life in human. This therapy might also reduce the severity of any type of acute/chronic disease (auto-immune related and inflammatory disorders) progression rate and can be used in both before and after the manifestation of any disease

symptoms in healthy, unhealthy, and ill peoples such as many thyroid disorders such hyperthyroidism, Goiter, Thyroid nodules, Thyroid cancer, Thyroid hormone resistance, Hashimoto's thyroiditis, Anaplastic Thyroid Cancer, Hypothyroidism, De Quervain's Thyroiditis, Medullary Thyroid Cancer, Follicular Thyroid Cancer, Papillary Thyroid Cancer, Silent Thyroiditis, Graves' Disease, Thyroid Cancer, Hurthle Cell Thyroid Cancer, and Thyroiditis. Overall, the data suggested the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* in showed significant action on thyroid gland with respect to biomarkers, as a Complementary and Alternative Medicine (CAM). This test formulation also can be used against Lupus, Fibromyalgia, Addison Disease, Multiple Sclerosis, Myasthenia Gravis, Aplastic Anemia, Psoriasis, Rheumatoid Arthritis, Crohn's Disease, Vitiligo, Chronic Fatigue Syndrome and Alopecia Areata, as well as various inflammatory disorders such as Ulcerative Colitis, Dermatitis, Hepatitis, Diverticulitis, Mental Disorders, Parkinson's and Other Movement Disorders, Stroke and Transient Ischemic Attack (TIA), and in the improvement of overall health and quality of life.

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