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Research Article

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Antioxidant and Anti-Inflammatory Activities of Biofield Energy Treated Proprietary Test Formulation in Brain Tissues in Cecal Slurry, LPS and *E. Coli*-Induced Systemic Inflammatory Response Syndrome (SIRS) in *Sprague Dawley* Rats

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Abstract

The study was aimed to evaluate the antioxidant and anti-inflammatory activity of the Biofield Energy Treated Proprietary Test Formulation and Biofield Energy Treatment *per se* to the animals on Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome (SIRS) model in *Sprague Dawley* rats. In this experiment, different antioxidants biomarkers such as myeloperoxidase (MPO), superoxide dismutase (SOD), lipid peroxidase (LPO) and cytokines like interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-2 (MIP-2) were analysed using ELISA assay in brain homogenate. A test formulation was formulated including minerals (magnesium, zinc, calcium, selenium, and iron), vitamin C, B_6, E, B_{12}, D_3, β-carotene, cannabidiol isolate, and *Panax ginseng* extract. The component of the test formulation were divided into two parts; one section was defined as the untreated, while the other portion of each constituent and three group of animals received Biofield Energy Healing/Blessing Treatment remotely for about 3 minutes by Mr. Mahendra Kumar Trivedi, a renowned spiritual Energy Healer. The level of MPO was significantly ($p \leq 0.001$) reduced by 19.43%, 34.91%, 25.43%, 25.29% and 30.33% in the G5 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treated test formulation); G6 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* to animals from day -15); G7 (Cecal

Slurry, LPS and *E. coli* + Biofield Energy Treated test formulation from day -15); G8 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated/Blessed test formulation from day -15), and G9 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation) groups, respectively as compared to the untreated test formulation (G4) group. Moreover, the level of SOD was significantly increased by 45.02% ($p \leq 0.001$), 16.59%, and 35.99% ($p \leq 0.001$) in the G6, G7, and G9 groups, respectively as compared to G4 group. The level of TNF-α was significantly decreased by 12.66%, 46.92% ($p \leq 0.001$), 26.57% ($p \leq 0.001$), 23.22% ($p \leq 0.001$), and 54.28% ($p \leq 0.001$) in G5, G6, G7, G8, and G9 groups, correspondingly with reference to G4 group. Moreover, the level of IL-6 was significantly ($p \leq 0.001$) decreased by 37.51%, 20.28%, 21.55%, and 33.4% in the G6, G7, G8, and G9 groups, respectively as compared to the G4 group. Additionally, the level of MIP-2 was significantly ($p \leq 0.001$) reduced by 47.97%, 17.08%, 20.16% and 26.84% in the G6, G7, G8, and G9 groups, respectively as compared to the G4 group. Together, the data imply the antioxidant and anti-inflammatory potential of the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* along with preventive measure on the animal with respect to various inflammatory conditions that might be beneficial various types of systemic inflammatory disorders specially sepsis, trauma, septic shock or any types of injuries. Therefore, the results described a significant reduction of inflammation-related disease progression rate and its complications in the preventive maintenance groups (*viz*. G6, G7, G8, and G9).

Introduction

Brain swelling or inflammation is an urgent clinical problem accompany with ischemic stroke, brain haemorrhage and traumatic brain injury. It occurs due to failure of membrane transporters and the blood-brain barrier (BBB), resulting in combination of cytotoxic, ionic and vasogenic edema. Brain swelling is also seen in acute liver failure, anoxic brain injury and toxin exposure [1]. Microglia plays a vital role in brain inflammation by the release of pro-inflammatory cytokines and with ageing [2]. Microglia, an immune cells of the brain, is constantly survey the microenvironment under physiological conditions. However, activated microglia can increase the expression of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) in brain tissue [3]. Several cytokines (TNF-α, TGF -β) and interleukins (IL-1, IL-4, IL-6, IL-8, and IL-18) are responsible for the development of various inflammatory pathologies of various vital systems such as brain, cardiac, renal, lymphatic, etc. [4]. MIP-2 is produced multiple cells like macrophages, epithelial cells, monocytes, and hepatocytes, in response to infection or injury. It is regulated by multiple factors like by signalling through Toll-like receptor 2 (TLR2), TLR3, and TLR4 in response to diverse pathogens [5], and in response to infections or injury by the activation of p38 mitogen-activated-protein-kinase-dependent signalling pathway [6]. Superoxide dismutases (SODs) is a very important antioxidant enzyme and also acts as a good therapeutic agent against reactive oxygen species-mediated diseases [7]. Proinflammatory cytokines affect nearly all tissues and organ systems.

Thus, in order to study the change in brain cytokines in presence of Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome model in Sprague Dawley rats, a novel proprietary test formulation was designed with minerals (magnesium, zinc, selenium, calcium, iron), essential vitamins (cyanocobalamin, ascorbic acid, pyridoxine HCl, vitamin E, and cholecalciferol), and nutraceuticals (β-carotene, cannabidiol isolate (CBD), Ginseng). The important vitamins and minerals incorporated in this proprietary formulation have significant functional role to maintain normal physiological balance [8, 9]. Besides, cannabidiol itself has wide range of pharmacological profile and has been reported to role in different disorders [10, 11],

while ginseng extract is regarded as the one of the best immune booster for overall immunity [12]. The current research work was undertaken to investigate the antioxidant and anti-inflammatory potential of the Biofield Energy Treated Proprietary Test Formulation and Biofield Energy Treatment *per se* to the animals on Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome model in Sprague Dawley rats.

"Biofield Therapy" has been reported with outstanding effects against various disorders, and got a position as one of the best Complementary and Alternative Medicine (CAM) treatment approach [13-15]. National Center for Complementary/Alternative Medicine (NCCAM) suggested CAM with several clinical benefits with reference to conventional medicine treatment approach [16]. National Centre of Complementary and Integrative Health (NCCIH) accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies such as deep breathing, natural products, Tai Chi, yoga, therapeutic touch, Johrei, Reiki, pranic healing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, movement therapy, hypnotherapy, relaxation techniques, mindfulness, special diets, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems [17, 18]. The Trivedi Effect®-Consciousness Energy Healing was scientifically reported on various disciplines such as nutraceuticals [19], agriculture science [20], cardiac health [21], materials science [22, 23], antiaging [24], Gut health [25], pharmaceuticals [26], overall human health and wellness. In this study, the authors want to evaluate the effect of the Biofield Energy Treatment (the Trivedi Effect®) on the given novel test formulation and Biofield Energy Treatment *per se* to the animals on brain biomarkers in presence of Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome model in in *Sprague Dawley* rats using standard ELISA assay.

Material and Methods

Chemicals and Reagents

Pyridoxine hydrochloride (vitamin B_6), zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, provit A) were purchased from TCI, Japan. Cyanocobalamin (vitamin B_{12}), calcium chloride, vitamin E (Alpha-Tocopherol), cholecalciferol (vitamin D_3), iron (II) sulfate, and carboxymethyl cellulose sodium were procured from Sigma-Aldrich, USA. Ascorbic acid (vitamin C) and sodium selenate were obtained from Alfa Aesar, India. *Panax ginseng* extract and Cannabidiol Isolate were obtained from Panacea Phytoextracts, India and Standard Hemp Company, USA, respectively. Dexamethasone was obtained from Clear synth, India. For the estimation of brain antioxidant and inflammatory biomarker panel, such as myeloperoxidase (MPO), superoxide dismutase (SOD), lipid peroxidation (LPO), tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2) were procured from CUSABIO, USA using specific ELISA kits.

Maintenance of Animal

Randomly breed male Sprague Dawley (SD) rats with body weight ranges from 200 to 300 gm 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 10-12 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

Each ingredient of the novel test formulation was divided into two parts. One part of the test compound did not receive any sort of treatment and were defined as the untreated or control sample. The 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 laboratory conditions for ~3 minutes. Besides, three group of animals also received Biofield Energy Healing Treatment (known as the Trivedi Effect®) by Mr. Mahendra Kumar Trivedi under similar laboratory conditions for ~3 minutes. The Blessing (prayer)/Treatment was given to the test items/animals (present in the laboratory of Dabur Research Foundation, near New Delhi, India), remotely from USA for about 3 minutes *via* online web-conferencing platform. 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 for ~3 minutes treatment, under the same laboratory conditions. The "sham" healer did not has any knowledge about the Biofield Energy Treatment. The Biofield Energy Treated animals were also taken back to experimental room for further proceedings

Experimental 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 (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na); G3 as reference item (Cecal Slurry, LPS and *E. coli* + Dexamethasone); G4 includes Cecal Slurry, LPS and *E. coli* along with untreated test formulation; G5 as Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation; G6 group includes Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15; G7 as Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* animals plus the untreated test formulation. Dosing for groups G7 and G8 were started on Day -15 and continued till end of the experiment. However, Group G1 to G5 and G9 animals were dosed with respective formulations from Day 1 and continued till the end of the experiment. Group G6 animals received Biofield Energy Treatment on Day-15 and were not dosed throughout the experimental period. At the end of the experimental period (8 weeks treatment), the animals were sacrifice and brain were collected, homogenised, and the supernatant subjected for estimation of antioxidants (MPO, SOD, and LPO) and cytokines (TNF alpha, IL-6, MIP-2).

Induction of Systemic Inflammatory Response Syndrome (SIRS) Model

A combination model of sepsis was developed in SD rats by administering Cecal slurry (from donor animals, intraperitoneally, at the dose of 400 mg/kg) in combination with LPS (at the dose of 100 µg/animal) and *E. coli* [*Escherichia coli*; 0.2 mL (2M CFU)/animal]). The animals were monitored for various parameters for up to 56 days after disease (SIRS) induction. Ten Donor (~20 weeks old) rats were anesthetized. A midline laparotomy was performed on them and the cecum was extruded. A 0.5 cm incision was made on the anti-mesenteric surface of the cecum, and the cecum was squeezed to expel the feces. The feces from different donor animals was collected and weighed. Immediately after collection, the feces were pooled, diluted 1:3 with 5% dextrose solution and filtered to get a homogeneous suspension. Bacterial viability in the cecal slurry was analyzed. Cecal slurry prepared from donor rats was injected intraperitoneally into experimental rats (G2 to G9) at the dose of 400 mg/kg within 2 hours of preparation. After 3 hours, lipopolysaccharide (LPS) at the dose of 100 µg/animal, and gram-negative viable

bacteria such as *E. coli* [0.2 mL (2M CFU)/animal] were injected, intraperitoneally (G2 to G9)

Preparation of Sample for the Estimation of Antioxidant and Cytokines

With the continued treatment to the respective groups of 8th week of the experimental period, all the animals were sacrificed, brain were collected, homogenized and subjected for the estimation of antioxidants and cytokines. The tissue 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 of cytokines during final calculations.

Estimation of Antioxidants and Cytokine Levels

The brain from all the groups was subjected for the estimation of level of antioxidants such as MPO (CSB-E08722r), SOD (706002), and LPO (700870) and cytokines such as TNF-α (CSB-E11987r), IL-6 (CSB-E04640r), and MIP-2 (CSB-E07419r). All the biomarker panel 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 antigen and antibody in sandwich manner assay.

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

Assessment of Antioxidants in Brain Homogenate

Estimation of Myeloperoxidase (MPO)

Myeloperoxidase (MPO), was estimated in the presence of the test formulation and the data is graphically presented in Figure 1. The data suggested that the disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) + 0.5% CMC) group (G2) showed value of MPO as 6.23 ± 0.33 ng/mL, which was increased by 90.27% as compared with the normal control (G1, 3.28 ± 0.08 ng/ mL). However, positive control (Dexamethasone) treatment (G3) showed the level of MPO in brain *i.e.* 5.37 ± 0.49 ng/mL, which was decreased by 13.9% as compared to the G2 group. The level of MPO in brain tissues was decreased by 8.89% and 2.49% in the G6 group includes Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15 and G9 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* plus untreated test formulation from day -15) groups, respectively as compared to the disease control (G2) group. On the other hand, the level of MPO was significantly ($p \leq 0.001$) reduced by 19.43%, 34.91%, 25.43%, 25.29% and 30.33% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group. Myeloperoxidase (MPO) is an inflammatory enzyme and therapeutic target for attenuating oxidative damage and neuro-inflammation in ischemic stroke in brain tissues. It is highly expressed in different inflammatory cells, like neutrophils, activated microglia, monocytes/macrophage, astrocytes, neurons, etc. [27]. Polymorphism of MPO enzyme may associated with the severity of brain damage [28]. Overall, in this experiment the Biofield Energy Treated test formulation reduced the level of MPO in the brain tissues, which could be helpful for the management of oxidative stress and inflammatory conditions.

Estimation of Superoxide Dismutase (SOD)

The effect of the test formulation and Biofield Energy Treatment *per se* was assessed by estimating the level of brain superoxide dismutase (SOD), and the results are graphically presented in the Figure 2. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) + 0.5% CMC) group (G2) showed value of SOD as 1.66 ± 0.09 U/mL, which was decreased by 9.12% as compared to the normal control group *i.e.,* 1.83 ± 0.14 U/mL.

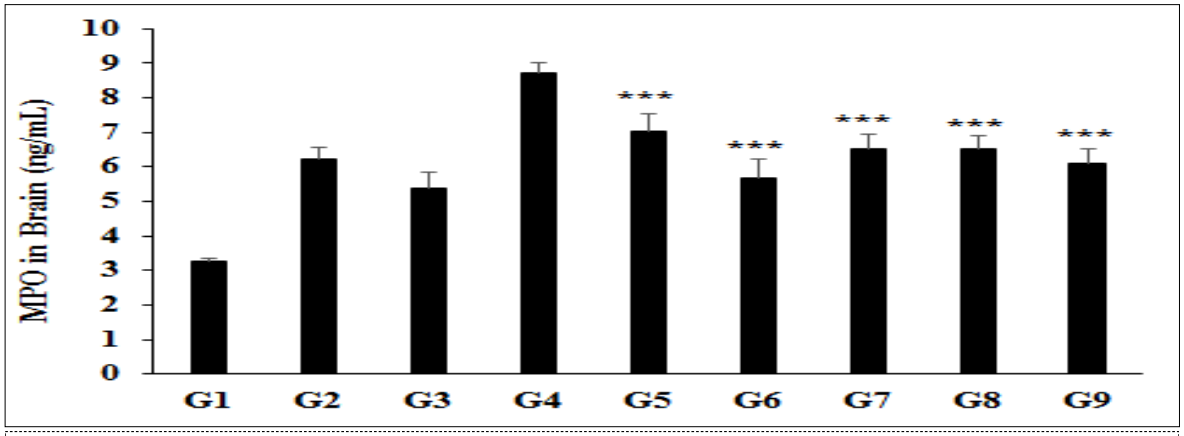

Figure 1. The effect of the test formulation on the level of brain myeloperoxidase (MPO) in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na); G3 as reference item (Cecal Slurry, LPS and *E. coli* + Dexamethasone); G4 includes Cecal Slurry, LPS and *E. coli* along with untreated test formulation; G5 as Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation; G6 group includes Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15; G7 as Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* animals plus the untreated test formulation. Values are presented as mean ± SEM (n=6-9). ***$p \leq 0.001$ *vs.* G4.

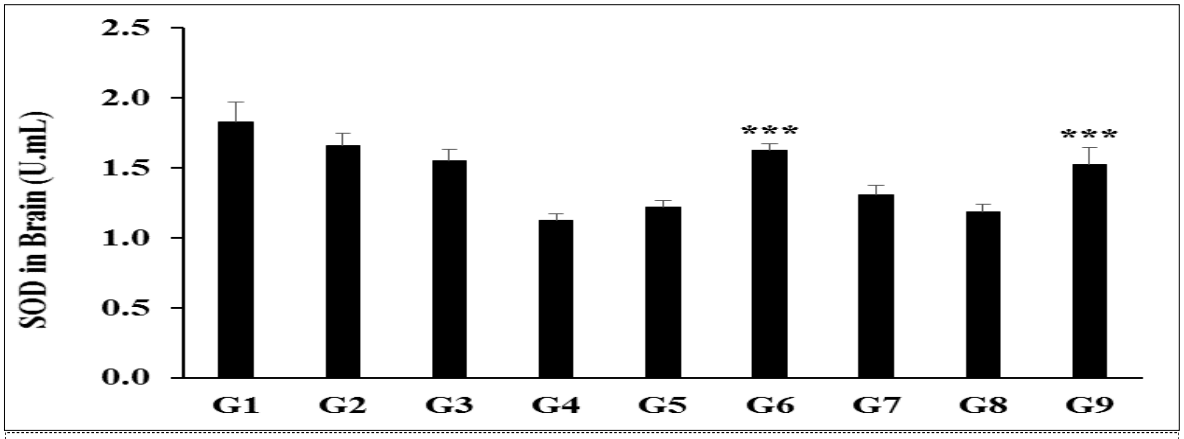

Figure 2. The expression level of brain superoxide dismutase (SOD) after administration of the Biofield Treated test formulation and Biofield Energy Healing/Blessing *per se* in Sprague Dawley rats. ***$p \leq 0.001$ *vs.* G4.

However, positive control (Dexamethasone) treatment (G3) showed the level of SOD in brain *i.e.* 1.55 ± 0.08 U/ mL. The level of SOD was increased by 8.77%, 45.02%, 16.59%, 6.11%, and 35.99% in the G5 (Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation); G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15), G7 as Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation from day -15; G8 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15), and G9 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the untreated test formulation group (G4). SOD are a group of metalloenzymes with diverse therapeutic activities in various physiological and pathological conditions such as inflammatory diseases, ischemia, aging, rheumatoid arthritis, neurodegenerative diseases, and cancer [29]. The enzyme can serve as an anti-inflammatory agent and can also prevent precancerous cell changes [30]. Overexpression of extracellular SOD can protects brain injury induced by chronic hypoxia [31]. Therefore, in this experiment the Biofield Energy Treated test formulation significantly increased the level of brain SOD, which could be beneficial inflammation and oxidative damage.

Estimation of lipid peroxidation (LPO)

The level of lipid peroxidation (LPO) end product in terms of malondialdehyde (MDA) was detected in all the experimental groups and the data are presented in Figure 3. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) and positive control (Dexamethasone) treatment (G3) groups showed value of MDA as 8.33 ± 0.64 µM and 12.26 ± 0.70 µM, respectively. The level of MDA was decreased by 4.35% in the G5 (Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation) group as compared to the untreated test formulation group (G4) group.

Assessment of Cytokines in Brain Homogenate

Estimation of Tumour Necrosis Factor Alpha (TNF-α)

The effect of the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* to the animals on the level of tumour necrosis factor alpha (TNF -α), and the results are shown in Figure 4. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of TNF-α as 69.66 ± 6.12 pg/mL, which was increased by 215.80% as compared with the normal control (G1, 22.06 ± 1.28 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) showed significant ($p \leq 0.001$) decreased TNF-α level by 41.11% *i.e.,* 41.02 ± 3.49 pg/mL as compared to the G2 group. The level of TNF-α was decreased by 25.59% and 35.91% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment per se to animals from day -15) and G9 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Further, the expression of TNF-α was significantly ($p \leq 0.001$) decreased by 12.66%, 46.92%, 26.57%, 23.22%, and 54.28% in the G5 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treated test formulation); G6 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* to animals from day -15); G7 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treated test formulation from day -15); G8 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15), and G9 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation) groups, respectively, as compared to the untreated test formulation group (G4). Tumor necrosis factor (TNF-alpha) plays a significant role in brain immune and inflammatory activities. Numerous evidence supports the role for TNF-alpha in injury induced by infectious, immune, toxic, traumatic, and ischemic stimuli. TNF-alpha promotes inflammation by stimulation of capillary endothelial cell proinflammatory responses [32]. It further causes neuro-inflammation in brain. Microglia is a major immune cells that involved in defense in the central

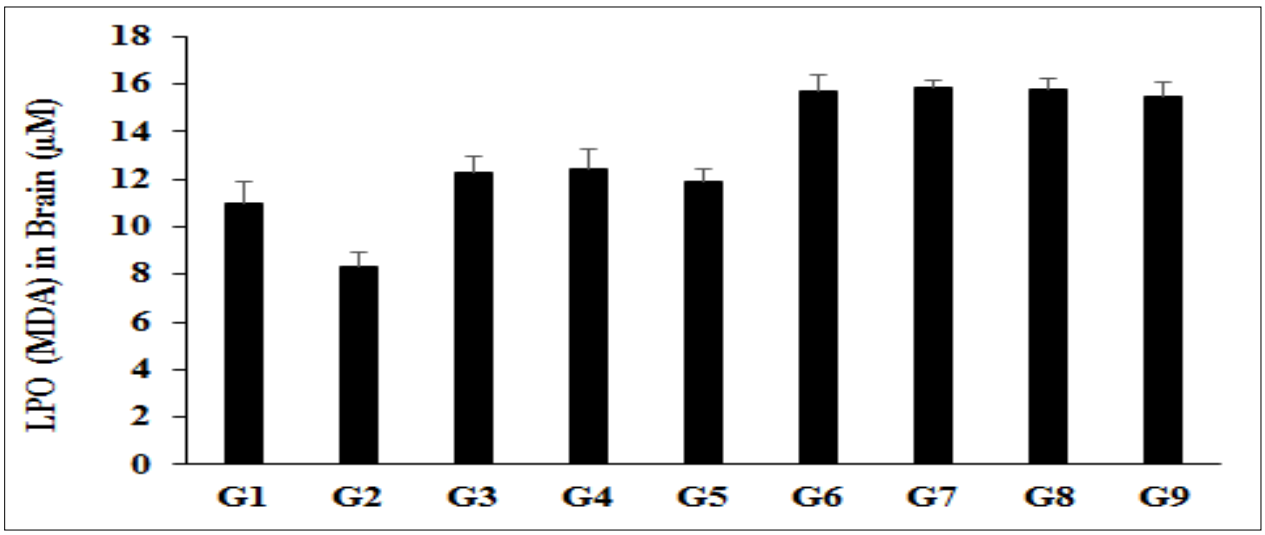

Figure 3. Expression of the level of brain lipid peroxidation (LPO) after administration of Biofield Treated test formulation and Biofield Energy Healing/Blessing *per se* to Sprague Dawley rats.

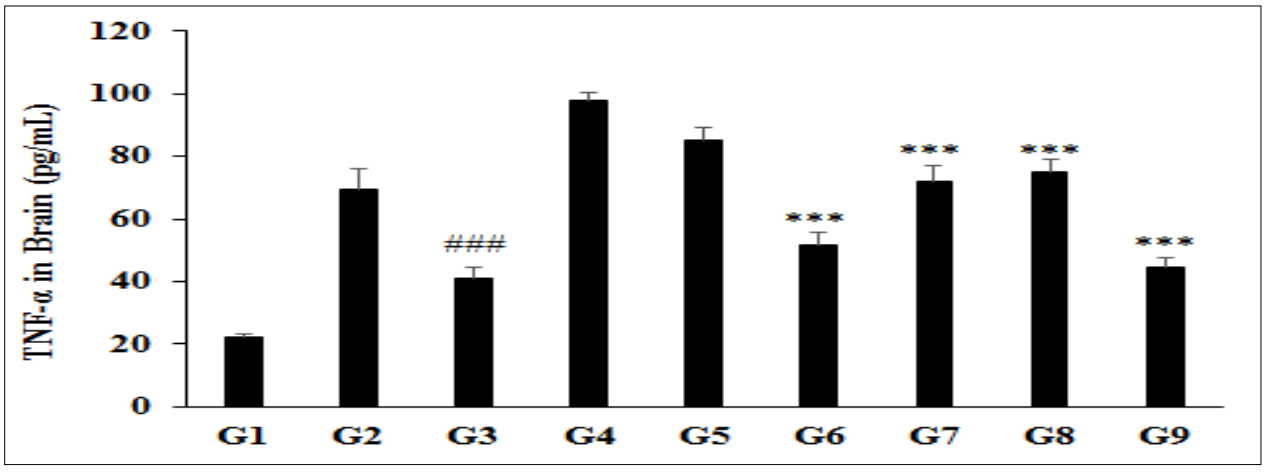

Figure 4. Expression of brain Tumour Necrosis Factor Alpha (TNF-α) after administration of Biofield Treated/Blessed test formulation and Biofield Energy Healing/Blessing *per se* to the Sprague Dawley rats. ###$p \leq 0.001$ *vs.* G2 and ***$p \leq 0.001$ *vs.* G4.

nervous system, and its activation leads to neuro-inflammation [33]. Activation of microglia cells causes acute brain ischemia, traumatic brain injury, etc. [34]. Therefore, Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment *per se* to the animals significantly reduced the level of TNF-*α*, which could be beneficial in the neuro-inflammation.

Estimation of Interleukin-6 (IL-6)

The effect of the test formulation and Biofield Energy Treatment *per se* on the level of brain interleukin-6, and the results are graphically presented in the Figure 5. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of IL-6 as 7.87 ± 0.79 pg/mL, which was increased by 43.95% as compared with the normal control (G1, 5.47 ± 0.28 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) showed the level of IL-6 *i.e.,* 7.24 ± 0.25 pg/mL. The level of IL-6 was significantly decreased by 21.86% and 16.73% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) and G9 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Further, the expression of IL-6 was significantly decreased by 7.42%, 37.51% ($p \leq 0.001$), 20.28% ($p \leq 0.001$), 21.55% ($p \leq 0.001$), and 33.4% ($p \leq 0.001$) in the G5 (Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation); G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15); G7 (Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation from day -15); G8 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15), and G9 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the untreated test formulation group (G4). The level of IL-6 is upregulated in various infection in CNS or injury or in a number of CNS diseases due to neuro-inflammation. IL-6 levels in CSF were also significantly higher than plasma levels in patients with traumatic brain injury [35]. Further, recent study revealed that IL-6 polymorphism associated with severe traumatic brain injury [36]. Overall, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of IL-6, which could be suppressed the neuroinflammatory conditions in the CNS and simultaneously reduce the risks of inflammatory diseases specially in the brain.

Estimation of Macrophage Inflammatory Protein-2 (MIP-2)

Expression of brain macrophage inflammatory protein-2 (MIP-2) after administration of Biofield Treated test formulation and Biofield Blessing directly to the rats was detected in all the experimental groups and the data is presented in Figure 6. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of MIP-2 as 4591.13 ± 401.95 pg/mL, which was increased by 199.83% as compared with the normal control (G1, 1531.27 ± 67.27 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) showed decreased brain MIP-2 level by 29.69% *i.e.,* 3228.11 ± 346.83 pg/mL as compared to the G2 group. The level of MIP-2 was decreased by 4.75%, 46.13%, 14.14%, 17.34%, and 24.26% in the G5 (Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation); G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15); G7 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treated test formulation from day -15); G8 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15), and G9 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Similarly, MIP-2 level was significantly decreased by 8%, 47.97%, 17.08%, 20.16% and 26.84% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group. MIP-2 is one of the

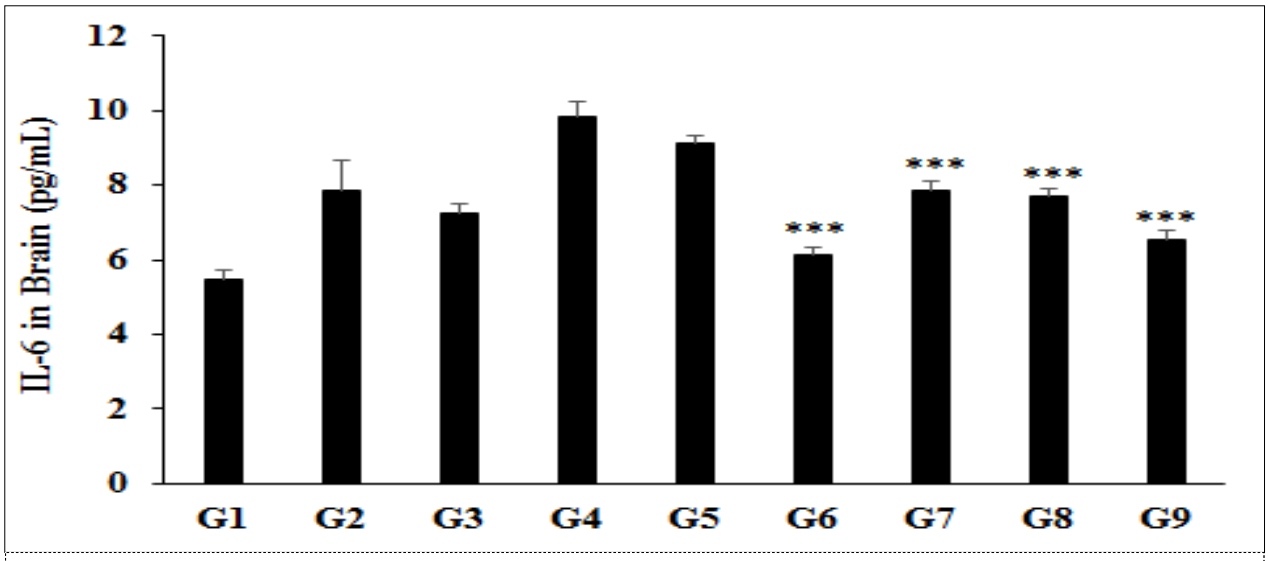

Figure 5. Expression of brain interleukin-6 (IL-6) after administration of Biofield Treated test formulation and Biofield Blessing *per se* to the Sprague Dawley rats. ***$p \leq 0.001$ *vs.* G4.

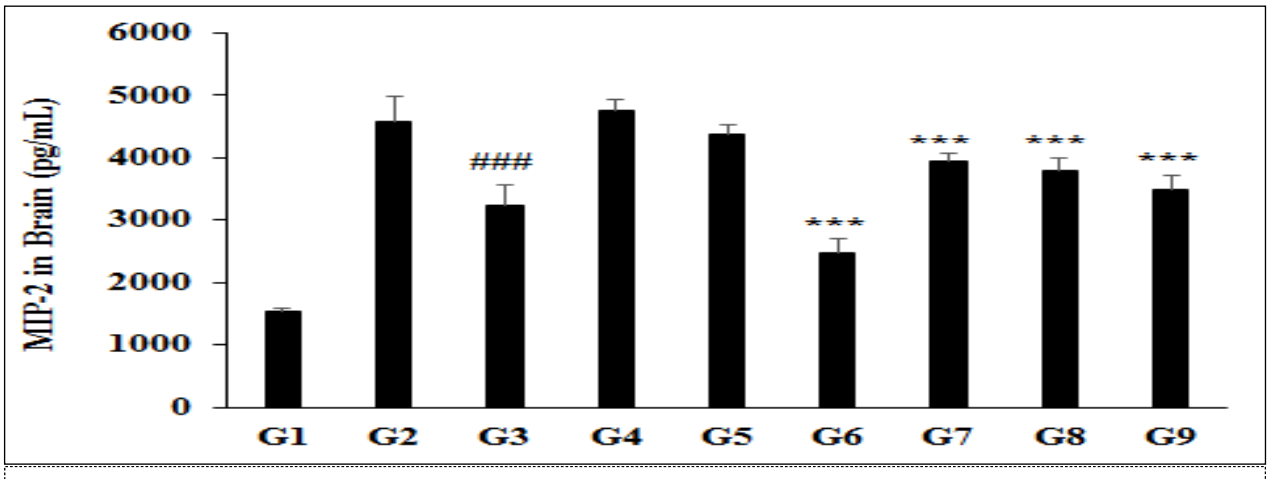

Figure 6. Expression of brain macrophage inflammatory protein-2 (MIP-2) after administration of Biofield Treated test formulation and Biofield Blessing directly to the Sprague Dawley rats. ###$p \leq 0.001$ *vs.* G2 and ***$p \leq 0.001$ *vs.* G4.

chemokine is produced in response to any types of infection or injury. For example, in liver injury the activated Kupffer cells are known as the major source of MIP-2 and causes liver inflammation [37]. In brain injury or inflammation, astrocytes is a prime source of chemokine (MIP-2), that plays an important function for induction of inflammation in the CNS [38]. As per literature report, one of the possible reason for brain inflammation is the polymorphonuclear neutrophils (PMNs) that mediates the brain inflammation followed by hypoxia/reoxygenation (H/R). They also studied the MIP-2 mRNA expression and found that H/R upregulated MIP-2 gene expression [39]. Taken together, our data suggest that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of MIP-2 in brain tissues, which could prevent the brain neuro-inflammation.

Experiment includes four preventive maintenance groups (G6, G7, G8 and G9). The findings showed the significant slowdown of inflammation-related symptoms and also reduced the chances of disease susceptibility. All-inclusive, it indicate that the Trivedi Effect® was found to be most effective and benefited to protect different kinds of diseases and also improve the overall health and quality of life.

Conclusions

The levels of antioxidants and cytokines in brain tissues were estimated and compared with respect to the disease control (G2) as well as untreated test formulation (G4) groups. The level of MPO was significantly decreased by 34.91%, 25.43%, 25.29% and 30.33% in the G6, G7, G8 and G9 groups, respectively with reference to the untreated test formulation (G4) group. The level of SOD was significantly increased by 45.02% and 35.99% in the G6 and G9 groups, respectively with reference to the untreated test formulation (G4) group. Moreover, the level of TNF-α was significantly reduced by 46.92%, 26.57%, 23.22%, and 54.28% in the G6, G7, G8, and G9 groups, respectively as compared to the G4 group. Additionally, IL-6 was significantly reduced by 37.51%, 20.28%, 21.55%, and 33.4% in G6, G7, G8, and G9 groups, respectively with reference to G4. Further, MIP-2 was significantly decreased by 47.97%, 20.16%, and 26.84% in the G6, G8, and G9 groups, respectively with reference to G4 group. Altogether, the Biofield Energy Treated test formulation and Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed significant results with respect to different inflammatory biomarkers (cytokines) in the preventive maintenance group, G6 as well as other preventive maintenance groups (G7, G8, and G9) in Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome model rat model study. It also helped to slowdown the inflammatory disease progression and disease-related complications. The study data showed that Biofield Energy Treated Test formulation and Biofield Energy Treatment *per se* would be one of the best treatment strategies to prevent the manifestation of diseases. Thus, the Biofield Energy Treatment might act as a preventive maintenance therapy to maintain and improve the overall health and quality of life and simultaneously reduce the severity of acute/chronic diseases. The test formulation can also be used against rheumatoid arthritis (RA), fibromyalgia, aplastic anaemia, Addison disease (AD), multiple sclerosis, myasthenia gravis, psoriasis, Crohn's disease, ulcerative colitis, dermatitis, hepatitis, Parkinson's, stroke, etc.

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