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Evaluation of Anti-inflammatory Activity after Treatment with the Biofield Energy Treated Proprietary Test Formulation on Combination of Cecal Slurry, LPS and *E. Coli* Induced Systemic Inflammatory Response Syndrome (SIRS) in Sprague Dawley Rats

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Abstract

The aim of this study was to evaluate the anti-inflammatory response 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 using serum inflammatory biomarkers. In this experiment, various inflammatory biomarkers such as monokine induced by gamma interferon (MIG), regulated on activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein-2 (MIP-2), matrix metallopeptidase 9 (MMP-9), troponin-1, procalcitonin, fibrinogen degradation product (FDP) were analysed using ELISA assay. A test formulation was formulated including minerals (magnesium, zinc, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, vitamin E, cyanocobalamin, and cholecalciferol), *Panax ginseng* extract, β-carotene, and cannabidiol isolate. The constituents of the test formulation were divided into two parts; one section was defined as the untreated test formulation, while the other portion of the test formulation and three group of animals received Biofield Energy Healing Treatment remotely for about 3 minutes by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The results showed that the level of MIG was significantly ($p \leq 0.01$) reduced by 48.57%, 56.14%, 47.95%, 51.92%, and 47.83% 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 disease control (G2) group.

Additionally, the level of regulated on activation, normal T cell expressed and secreted (RANTES) was significantly ($p \leq 0.001$) decreased by 47.35%, 46.32%, 40.73%, 50.47%, and 39.96% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. The level of macrophage inflammatory protein-2 (MIP-2) was significantly ($p \leq 0.001$) decreased by 91.74%, 93.54%, 92.70%, 94.46%, and 93.80% in the G5, G6, G7, G8, and G9 groups, as compared to the G2 group. The level of MMP-9 was significantly ($p \leq 0.001$) decreased by 18.74%, 35.54%, 26.48%, 38.16%, and 22.52% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. Moreover, the level of troponin-1 was decreased by 28.53%, 28.50%, 14.14%, 30.68%, and 36.34% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group. Additionally, the level of procalcitonin was significantly ($p \leq 0.001$) decreased by 27.27%, 51.73%, 46.40%, 54.65%, and 39.08% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. Further, the level of fibrinogen degradation product (FDP) was significantly ($p \leq 0.001$) decreased by 66.7%, 62.5%, 31.5%, 51.6% and 40.1% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. Overall, the data suggested anti-inflammatory potentials 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 showed the significant slowdown the inflammation-related disease progression and its complications/symptoms 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 control group.

Keywords: Biofield treatment; Inflammatory biomarkers; The Trivedi effect®; ELISA; SIRS

Abbreviations: SIRS: Systemic Inflammatory Response Syndrome; FDP: Fibrinogen Degradation Products; CD: Crohn's Disease; UC: Ulcerative Colitis; CBD: Cannabidiol Isolate; CAM: Complementary and Alternative Medicine; SD: Sprague Dawley

Introduction

Systemic inflammatory response syndrome (SIRS) is a complex pathophysiologic defense response of the body to a noxious stressor such as infection, trauma, burns, pancreatitis, surgery, acute inflammation, ischemia or reperfusion, or malignancy

or any others injuries [1,2]. Sepsis is an infection which can considered a systemic inflammatory response. Clinically, the Systemic Inflammatory Response Syndrome (SIRS) is identified by two or more symptoms including fever or hypothermia, tachycardia, tachypnoea and change in blood leucocyte count [3]. Sepsis is a systemic inflammatory response to a confirmed or suspected infection. The development from sepsis to septic shock represents a continuum with increasing mortality. Research in the last two decades explored that the inflammatory process is play a major role in the mechanism of different vital systems pathologies [4]. Proinflammatory cytokines affect nearly all tissues and organ systems. RANTES is of broad clinical importance in an array of human diseases including AIDS, cancer, atherosclerosis, asthma, transplantation, and autoimmune diseases such as arthritis, diabetes and glomerulonephritis [5]. The level of fibrin and fibrinogen degradation products (FDP) are increased in case of malignancy, collagen disorders, infections and other conditions [6]. Serum procalcitonin is one of the biomarker for the diagnosis of sepsis, any others infectious or inflammatory conditions [7,8]. Metalloproteinases expression was associated with the presence of erosions, architectural tissue changes, and inflammatory infiltration. Overexpression of metalloproteinases causes development of inflammatory disorders like Crohn's disease (CD) and ulcerative colitis (UC), etc. [9,10].

Thus, in order to study the change in serum biomarkers in presence of Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome model in Sprague Dawley rats, a novel test formulation was designed with the combination of vital minerals (selenium, zinc, iron, calcium, and magnesium), essential vitamins (cyanocobalamin, ascorbic acid, pyridoxine HCl, vitamin E, and cholecalciferol), and nutraceuticals (β-carotene, Ginseng, cannabidiol isolate (CBD)). All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological roles [11]. Besides, cannabidiol itself has wide range of pharmacological profile and has been reported to role in different disorders [12,13], while ginseng extract is regarded as the one of the best immune booster for overall immunity [14]. The present study was aimed to evaluate the 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 using serum biomarkers (cytokines). Biofield Energy Healing Treatment has been reported with significant effects against various disorders, and defined as one of the best Complementary and Alternative Medicine (CAM) treatment approach [15-17].

National Center for Complementary/Alternative Medicine (NCCAM) recommended CAM with several clinical benefits as compared with the conventional treatment approach [18]. 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, hypnotherapy, special diets, relaxation techniques, movement therapy, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems [19,20]. The Trivedi Effect®-Consciousness Energy Healing was scientifically reported on various disciplines such as nutraceuticals [21], agriculture science [22], cardiac health [23], materials science [24,25], antiaging [26], Gut health [27], pharmaceuticals [28], overall human health and wellness. In this study, the authors sought to study the impact of the Biofield Energy Treatment (the Trivedi Effect®) on the given novel test formulation and Biofield Energy Treatment *per se* to the animals on serum biomarkers in presence of Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome model in Sprague Dawley Rats for the estimation of monokine induced by gamma interferon (MIG), regulated on activation, normal T cell expressed and secreted (RANTES), MIP-2, MMP-9, Troponin-1, Procalcitonin, Fibrinogen Degradation Product (FDP) using standard ELISA assay.

Material and Methods

Chemicals and Reagents

Pyridoxine hydrochloride (vitamin B6), zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, provit A) were purchased from TCI, Japan. Cyanocobalamin (vitamin B12), calcium chloride, vitamin E (Alpha-Tocopherol), cholecalciferol (vitamin D3), 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 serum biomarker panel, specific ELISA kits were used such as for the detection of MIG, RANTES, MIP-2, MMP-9, Troponin-1, Procalcitonin, Fibrinogen Degradation Product were procured from CUSABIO, USA.

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

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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 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/Blessing (prayer) was given to the samples or animals remotely 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 blood was collected and separate serum subjected for the estimation of MIG, RANTES, MIP-2, MMP-9, Troponin-1, Procalcitonin, Fibrinogen Degradation Product.

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 Serum Biomarkers

With the continued treatment to the respective groups of 8th 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 of cytokines during final calculations.

Estimation of Inflammatory Biomarkers in Serum

The serum from all the groups was subjected for the estimation of the level of inflammatory biomarkers such as MIG (CSB-EL006252RA), RANTES (CSB-E07398r), MIP-2 (CSB-E07419r), MMP-9 (CSB-E08008r), Troponin-1 (CSB-E08594r), Procalcitonin (CSB-E13419r), Fibrinogen Degradation Product (CSB-E07942r). All the serum 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.

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Results and Discussion

Estimation of Monokine Induced by Gamma Interferon (MIG) in Serum

Monokine induced by Gamma Interferon (MIG) in serum was estimated in the presence of the test formulation and the data were graphically shown 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 MIG as 351.24 ± 84.13 pg/mL, which was increased by 208.72% as compared with the normal control (G1, 113.77 ± 14.56 pg/mL). However, positive control (Dexamethasone) treatment (G3) showed the level of serum MIG i.e. 157.20 ± 16.29 pg/mL, which was significantly ($p \leq 0.01$) decreased by 55.24% as compared to the G2 group. The level of MIG was significantly decreased by 14.5%, 48.57% ($p \leq 0.01$), 56.14% ($p \leq 0.01$), 47.95% ($p \leq 0.01$), 51.92% ($p \leq 0.01$), and 47.83% ($p \leq 0.01$) in the G4 (Cecal Slurry, LPS and *E. coli* along with untreated test formulation); 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 disease control (G2) group. On the other hand, the level of MIG was reduced by 39.85%, 48.7%, 39.12%, 43.77%, and 38.98% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). Based on the existed information the level of MIG is higher in case of haemophagocytic lymphohistiocytosis (HLH), Epstein-Barr virus (EBV) infection, anaplastic large cell lymphoma, etc. [29]. Overall, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* reduced the level of MIG, which might be helpful for the management of various inflammatory disorders.

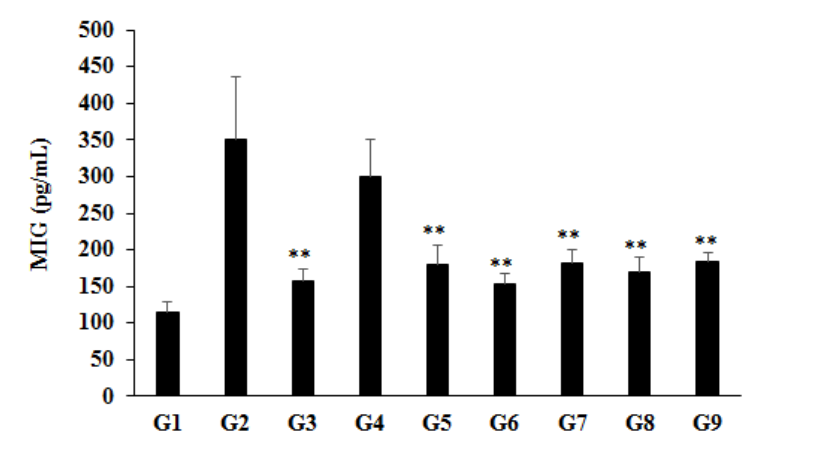

Figure 1: The effect of the test formulation (Biofield Treated and Untreated) and Biofield Energy Healing/Blessing *per se* on the level of serum monokine induced by gamma interferon (MIG) in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS 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 + Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation. Values are presented as mean ± SEM (n=6-9). ** $p \leq 0.01$ *vs*. G2.

Estimation of Serum regulated on activation, normal T cell expressed and secreted (RANTES)

RANTES is widely clinical importance in an array of human diseases including asthma, atherosclerosis, arthritis, glomerulonephritis, diabetes, AIDS, and cancer, etc. [5]. Estimation the level of serum regulated on activation, normal T cell expressed and secreted (RANTES) in Sprague Dawley rats after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing/Blessing *per se*, and the results were graphically shown in Figure 2. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) + 0.5% CMC) group (G2) showed value of RANTES as 3.32 ± 0.38 ng/mL, which was increased by 233.80% as compared with the normal control (G1, 0.99 ± 0.10 ng/mL). However, positive control (Dexamethasone) treatment (G3) showed the level of serum RANTES i.e., 1.59 ± 0.08 ng/mL, which was significantly ($p \leq 0.001$) decreased by 52.13% as compared to the G2 group. The level of RANTES was significantly ($p \leq 0.001$) decreased by 34.58%, 47.35%, 46.32%, 40.73%, 50.47%, and 39.96% in the G4 (Cecal Slurry, LPS and *E. coli* along with untreated test formulation); G5 (Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test

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formulation); G7 (Cecal Slurry, LPS and *E. coli* + 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 with reference to disease control group (G2). On the other hand, the level of RANTES was reduced by 19.45%, 17.87%, 9.32%, 24.23%, and 8.15% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). Therefore, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* reduced the level of RANTES, which could be beneficial in the inflammatory disease conditions.

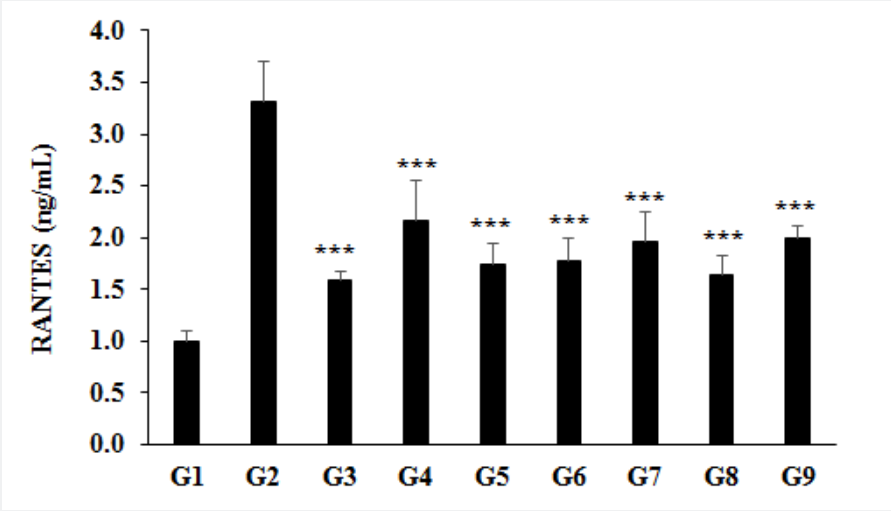

Figure 2: Estimation the level of serum regulated on activation, normal T cell expressed and secreted (RANTES) in Sprague Dawley rats after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing/Blessing *per se*. G1 as normal control (vehicle, 0.5% w/v 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* + Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation. Values are presented as mean ± SEM (n=6-9). ***$p \leq 0.001$ *vs.* G2.

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

The level of serum macrophage inflammatory protein-2 (MIP-2) was detected in all the experimental groups and is presented in Figure 3. The data showed that the disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of MIP-2 as 1136.41 ± 90.76 pg/mL, which was increased by 1141.98% as compared with the normal control (G1, 91.50 ± 4.48 pg/mL) group. While, the positive control (Dexamethasone) treatment (G3) was significantly ($p \leq 0.001$) decreased the level of MIP-2 by 89.51% i.e. 119.16 ± 8.46 pg/mL as compared to the G2 group. The level of MIP-2 was significantly ($p \leq 0.001$) decreased by 40.87%, 91.74%, 93.54%, 92.70%, 94.46%, and 93.80% in the G4 (Cecal Slurry, LPS and *E. coli* along with untreated test formulation); G5 (Cecal Slurry, LPS and *E. coli* along with the Biofield Energy Treated test formulation); G7 (Cecal Slurry, LPS and *E. coli* + 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 with reference to disease control group (G2). On the other hand, the level of MIP-2 was reduced by 86.03%, 89.08%, 87.65%, 90.63%, and 89.52% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). MIP-2-recruited and activated neutrophils can accelerate liver inflammation by releasing various inflammatory mediators [30]. Overall, here the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* has increased the level of MIP-2, which could be beneficial in the inflammatory symptoms.

Estimation of serum matrix metallopeptidase 9 (MMP-9)

Expression the level of serum matrix metalloproteinase 9 (MMP-9) after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing *per se* to procalcitonin in Sprague Dawley rats, and the results are graphically presented in the Figure 4. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of MMP-9 as 182.03 ± 17.77 pg/mL, which was increased by 28.99% as compared with the normal control (G1, 141.12 ± 8.99 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) showed a significant ($p \leq 0.001$) decreased serum MMP-9 level by 33.99% i.e., 120.17 ± 3.29 pg/mL as compared to the G2 group. The level of MMP-9 was significantly ($p \leq 0.001$) decreased by 22.5%, 18.74%, 35.54%, 26.48%, 38.16%, and 22.52% in the G4 (Cecal Slurry, LPS and *E. coli* along with untreated test

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formulation); G5 (Cecal Slurry, LPS and *E. coli* along with the 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 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 disease control group (G2). Besides, the level of MMP-9 was reduced by 16.82%, 5.13%, and 20.20% in the G6, G7, and G8 groups, respectively as compared to the untreated test formulation (G4). Matrix metalloproteinases as modulators of inflammation and its expression was increased in the inflammatory cells and modulates various inflammatory mediators like cytokines and chemokines in the inflamed tissues that regulate the movement of leukocytes at sites of infection or injury [31]. All-inclusive, the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of MMP-9, which could be beneficial to combat inflammatory disease conditions.

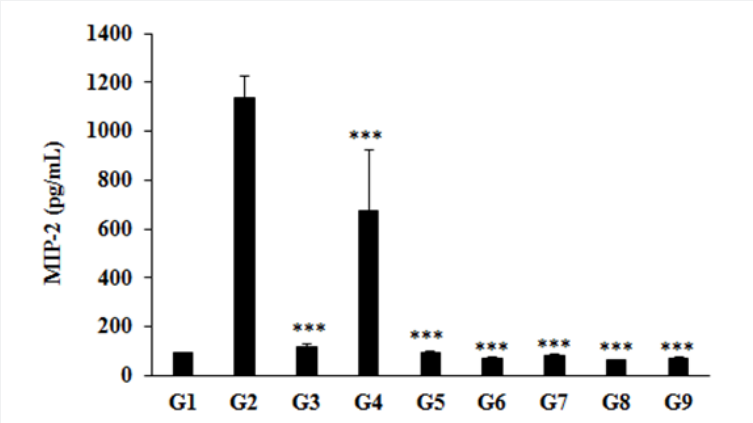

Figure 3: Expression the level of serum macrophage inflammatory protein-2 (MIP-2) after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing *per se* to procalcitonin in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease 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* + Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation. Values are presented as mean ± SEM (n=6-9). ***$p \leq 0.001$ *vs*. G2.

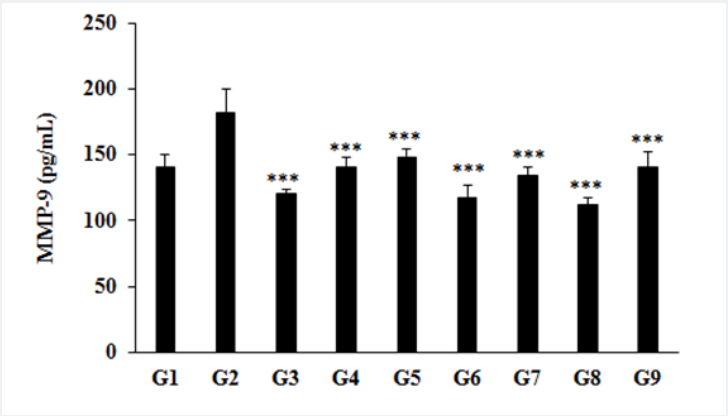

Figure 4: Expression the level of serum matrix metalloproteinase 9 (MMP-9) after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing *per se* to procalcitonin in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (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* + Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation. Values are presented as mean ± SEM (n=6-9). ***$p \leq 0.001$ *vs*. G2.

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Estimation of Serum Troponin-1

Expression the level of serum troponin-1 after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing *per se* to procalcitonin in Sprague Dawley rats, and the results are graphically presented in Figure 5. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of troponin-1 as 100.86 ± 8.72 pg/mL, which was increased by 83.87% as compared with the normal control (G1, 54.85 ± 2.81 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) showed a significant ($p \leq 0.001$) decreased serum troponin-1 level by 39.81% i.e., 60.71 ± 5.17 pg/mL as compared to the G2 group. The level of troponin-1 was significantly decreased by 22.46%, 22.43%, 6.85%, 24.79%, and 30.93% in the G5 (Cecal Slurry, LPS and *E. coli* along with the 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 test formulation from day -15), and G9 (Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation) groups, respectively with reference to disease control group (G2). Similarly, troponin-1 level was decreased by 28.53%, 28.50%, 14.14%, 30.68%, and 36.34% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). Based on the literature it has been suggest that troponin I can induces severe inflammatory responses [32]. Overall, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of troponin-1, which could regulate the inflammatory conditions and simultaneously reduce the risks of inflammatory diseases.

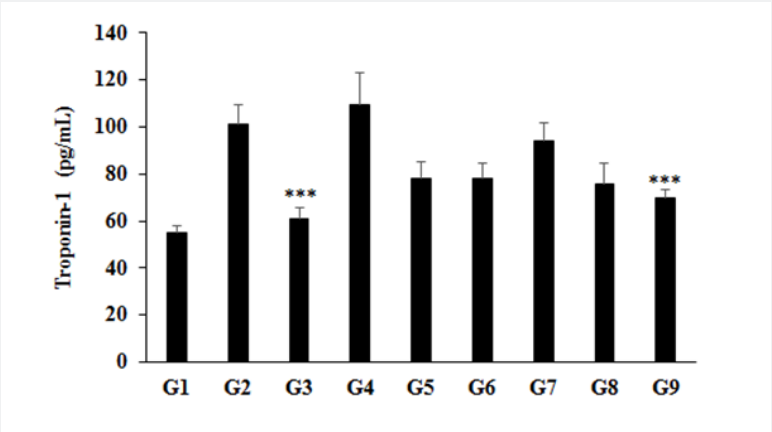

Figure 5: Expression the level of serum troponin-1 after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing *per se* to procalcitonin in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *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* + Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation. Values are presented as mean ± SEM (n=6-9). ***$p \leq 0.001$ *vs*. G2.

Estimation of Serum Procalcitonin

The level of serum procalcitonin was detected in all the experimental groups and the data are presented in Figure 6. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of procalcitonin as 924.56 ± 132.48 pg/mL, which was increased by 159.37% as compared with the normal control (G1, 356.46 ± 29.68 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) showed a significant ($p \leq 0.001$) decrease the level of serum procalcitonin by 46.08% i.e., 498.56 ± 28.14 pg/mL as compared to the G2 group. The level of procalcitonin was significantly ($p \leq 0.001$) decreased by 16.02%, 27.27%, 51.73%, 46.40%, 54.65%, and 39.08% in the G4 includes Cecal Slurry, LPS and *E. coli* along with untreated test formulation; 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 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 disease control group (G2). Similarly, procalcitonin level was decreased by 13.4%, 42.53%, 36.18%, 46%, and 27.46% in G5, G6, G7, G8, and G9 groups, correspondingly with reference to untreated test formulation (G4) group. The procalcitonin is a useful biomarker of several clinical conditions like systemic inflammation, infection, and

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sepsis [33]. Concentration of procalcitonin selectively increases in inflammatory conditions [34]. Overall, here the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* reduced the level of procalcitonin, which could be beneficial in the inflammatory symptoms.

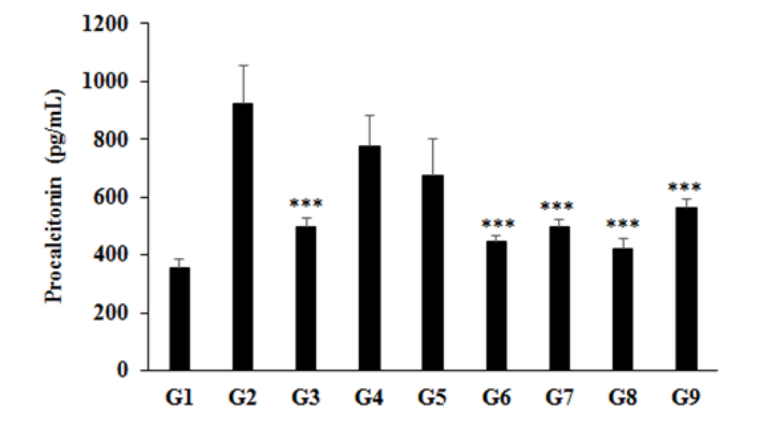

Figure 6: Expression the level of serum procalcitonin after administration of Biofield Treated/Untreated proprietary test formulation and Biofield Energy Healing *per se* to procalcitonin in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS 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* + Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation. Values are presented as mean ± SEM (n=6-9). ***$p \leq 0.001$ *vs*. G2.

Estimation of Serum Fibrinogen Degradation Product (FDP)

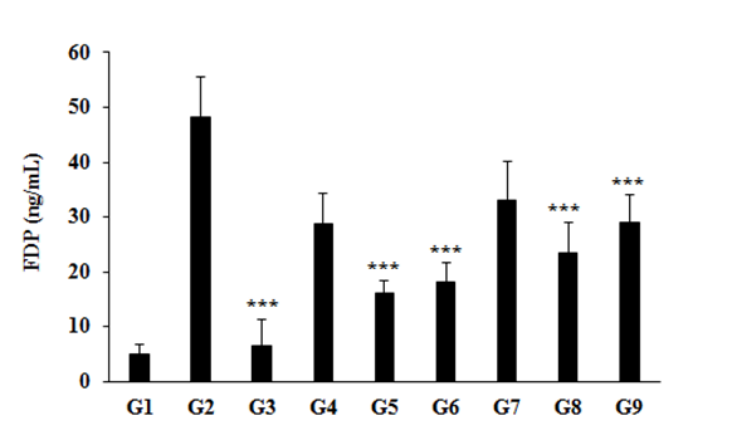

Figure 7: The effect of the test formulation on the level of serum Fibrinogen Degradation Product (FDP) in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na); G3 as reference item (Cecal Slurry, LPS and *E. coli* + 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* + Biofield Energy Treated test formulation from day -15; G8 group includes Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* + Biofield Energy Treated test formulation from day -15, and G9 group denoted Cecal Slurry, LPS and *E. coli* + Biofield Energy Treatment *per se* animals + untreated test formulation. Values are presented as mean ± SEM (n=6-9). ***$p \leq 0.001$ *vs*. G2.

The level of serum fibrinogen degradation product (FDP) was detected in all the experimental groups and the data are presented in Figure 7. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of FDP as 48.32 ± 7.17 ng/mL, which was increased by 870.8% as compared with the normal control (G1, 4.98 ± 1.74 ng/mL). Further, the positive control (Dexamethasone) treatment (G3) showed a significant ($p \leq 0.001$) decrease the level of serum FDP by 86.4% i.e., 6.57 ± 4.63 ng/mL as compared to the G2 group. The level of FDP was significantly decreased by 40.7%, 66.7% ($p \leq 0.001$),

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62.5% ($p \leq 0.001$), 31.5%, 51.6% ($p \leq 0.001$) and 40.1% ($p \leq 0.001$) in the G4 (Cecal Slurry, LPS and *E. coli* along with untreated test formulation); 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, correspondingly with reference to disease control group (G2). Similarly, FDP level was decreased by 43.8%, 36.8%, and 18.4% in the G5, G6, and G8 groups, respectively as compared to the untreated test formulation (G4). FDP are the components of blood produced by clot degeneration. In normal subjects, the plasma FDP levels are not detectable. When the levels are raised above 200 ng/mL, it can be detectable in the plasma. Besides, in response to inflammation, the body produces more fibrinogen and its degradation products [35]. Overall, here the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* reduced the level of FDP, which could be beneficial in the inflammatory symptoms. 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.

Conclusion

The level of inflammatory biomarkers such as monokine induced by gamma interferon (MIG), regulated on activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein-2 (MIP-2), matrix metallopeptidase 9 (MMP-9), Troponin-1, Procalcitonin, Fibrinogen Degradation Product (FDP) were estimated and compared with respect to the disease control (G2) as well as untreated test formulation group (G4). Serum MIG level was significantly reduced by 48.57%, 56.14%, 47.95%, 51.92%, and 47.83% in the G5 (Cecal Slurry, LPS and *E. coli* along with the 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 disease control (G2) group. RANTES was significantly decreased by 47.35%, 46.32%, 40.73%, 50.47%, and 39.96% in G5, G6, G7, G8, and G9 groups, correspondingly with reference to G2 group. Moreover, the level of MIP-2 was significantly decreased by 91.74%, 93.54%, 92.70%, 94.46%, and 93.80% in the G5, G6, G7, G8, and G9 groups, as compared to the G2 group. Additionally, MMP-9 was significantly decreased by 18.74%, 35.54%, 26.48%, 38.16%, and 22.52% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group.

Further, troponin-1 was decreased 28.53%, 28.50%, 14.14%, 30.68%, and 36.34% in G5, G6, G7, G8, and G9 groups, correspondingly with reference to untreated test formulation group (G4). Procalcitonin was significantly decreased by 27.27%, 51.73%, 46.40%, 54.65%, and 39.08% in G5, G6, G7, G8, and G9 groups, correspondingly with reference to G2 group. The level of FDP was significantly decreased by 66.7%, 62.5%, 31.5%, 51.6% and 40.1% in G5, G6, G7, G8, and G9 groups, correspondingly with reference to G2 group. All-inclusive, the Biofield Energy Treated test formulation and Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed fruitful results with respect to different inflammatory biomarkers 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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