MEDWIN PUBLISHERS
Committed to Create Value for Researchers

Journal of Natural & Ayurvedic Medicine
ISSN: 2578-4986

Anti-Inflammatory Activity of Biofield Energy Treated Proprietary Test Formulation on Cecal Slurry, LPS and *E. coli* Induced Systemic Inflammatory Response Syndrome (SIRS) Model in Sprague Dawley Rats

Trivedi MK[1], Branton A[1], Trivedi D[1] and Jana S[2]*

[1]Trivedi Global, Inc., Henderson, Nevada, USA

[2]Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India

***Corresponding author:** Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India, Email: publication@trivedisrl.com

Research Article

Volume 5 Issue 3

Received Date: July 10, 2021

Published Date: July 26, 2021

DOI: 10.23880/jonam-16000317

Abstract

The aim of this experiment was 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 (SIRS) model in Sprague Dawley rats. The parameters studies in this experiment includes tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2), matrix metallopeptidase 9 (MMP-9), fibrin degradation products (FDP), Substance P, inducible nitric oxide synthase (iNOS) 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 (prayer) remotely for about 3 minutes by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The level of TNF-α was significantly ($p \leq 0.001$) decreased by 46.94% and 55.91% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) group as compared to the disease control group (G2) and the untreated test formulation (G4), respectively. The level of IL-6 was significantly ($p \leq 0.001$) reduced by 51.44% and 42.92% in the G6 group as compared to the disease control (G2) group and untreated test formulation group, respectively. The level of MIP-2 was significantly reduced by 21.52% and 31.54% ($p \leq 0.001$) in the G6 group as compared to the G2 and G4 groups, respectively. The level of MMP-9 was significantly decreased by 40.13% ($p \leq 0.001$) and 20.92% in the G6 and G8 groups, respectively as compared to the G2 group. Moreover, the level of FDP was significantly ($p \leq 0.001$) decreased by 38.22% and 50.53% in the G6 group as compared to the G2 and G4 groups, respectively. The level of substance P was significantly ($p \leq 0.001$) decreased by 36.96% and 38.20% in the G6 group as compared to the G2 and G4, groups, respectively. The level of iNOS was significantly ($p \leq 0.001$) decreased by 35.41% in the G6 group as compared to the G4 group. Overall, the data suggested the 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* group (G6) comparatively with the disease control group.

Keywords: Biofield Treatment; Inflammatory Biomarkers; The Trivedi Effect®; Elisa; Sirs

Abbreviations: AD: Addison Disease; RA: Rheumatoid Arthritis; SIRS: Systemic Inflammatory Response Syndrome; NR: Nitric Oxide; FDP: Fibrin Degradation Products; MMP-9: Matrix Metallopeptidase 9; SEM: Standard Error of Mean; LPS: Lipopolysaccharide; SD: Sprague Dawley; IAEC :Institutional Animal Ethics Committee; NCCAM: National Center For Complementary/Alternative Medicine; CAM: Complementary And Alternative Medicine; CBDI: Cannabidiol Isolate; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; PMNs: Polymorphonuclear Neutrophils; MMPs; Matrix Metalloproteinases.

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]. Inflammatory syndrome to systemic inflammatory response syndrome (SIRS) are associated with the multi-organ dysfunction syndrome (MODS). Tumor necrosis factor alpha (TNF-α) is proinflammatory cytokine that may link inflammation to SIRS, that ultimately leads to MODS [5]. MIP-2 is one of the chemokine released from different cells like neutrophils, macrophages, hepatocytes, monocytes, etc. in response to infections or injury by the activation of p38 mitogen-activated-protein (MAP)-kinase-dependent signalling pathway [6].

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidase enzymes, responsible for tissue remodelling in both physiological and pathophysiological conditions [7]. Fibrin degradation products (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 [8,9]. The neuropeptide substance P (SP) is an 11 amino acid peptide distributed throughout the nervous system of human and animal species. SP has a potent neuroimmunomodulatory actions through mediation of neurokinin-1 receptor and proinflammatory effects *in vitro* and *in vivo*, and also influence many immune and inflammatory disorders [8,9]. There is increasing evidence that nitric oxide (NO) is an important factor in the pathogenesis of septic shock. According to sukahara T, et al. [10]. reported that the mRNA expression of inducible NO synthase (iNOS) has increased in both sepsis and SIRS cases, which measured in terms of polymorphonuclear neutrophils (PMNs) by reverse transcriptase polymerase chain reaction (RT-PCR) method.

Thus, in order to study the change in antioxidants and inflammatory biomarkers in lungs and liver 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 (CBDI). All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological roles [11,12]. Besides, cannabidiol itself has wide range of pharmacological profile and has been reported to role in different disorders [13,14]. while ginseng extract is regarded as the one of the best immune booster for overall immunity [15]. The present study was aimed to evaluate 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 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 [16-18]. National Center for Complementary/Alternative Medicine (NCCAM) recommended CAM with several clinical benefits as compared with the conventional treatment approach [19]. 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 [20,21]. The Trivedi Effect®-Consciousness Energy Healing was scientifically reported on various disciplines such as nutraceuticals agriculture science, cardiac health, materials science, antiaging, Gut health, pharmaceuticals, overall human health and wellness

[22-29]. In this study, the authors want to evaluate 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 liver 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. Estimation of anti-inflammatory and other vital biomarkers like TNF alpha, IL-6, MIP-2, MMP-9, FDP, Substance P, and iNOS in the liver homogenate the specific ELISA kits were procured from CUSABIO, USA.

Animal Welfare

All the animals were handled humanely with due regard for their welfare. Care of animals were complied with the Regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India. The test facility (Dabur Research Foundation, India) was registered (Registration No. 64/PO/RcBi/S/99/CPCSEA) for experiment of animals with the CPCSEA. The animals were procured using Institutional Animal Ethics Committee (IAEC) approved protocol (IAEC/42/533) and the husbandry conditions maintained as per CPCSEA recommendations.

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

The novel test formulation was consisted of zinc chloride, iron (II) sulfate, vitamin B_6, vitamin B_{12}, vitamin D_3, vitamin E (Alpha-tocopherol), sodium selenate, calcium chloride, ascorbic acid, beta carotene, *Panax ginseng* extract, cannabidiol and magnesium (II) gluconate. 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 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 test items/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 liver were collected, homogenised, and the supernatant subjected for estimation of TNF alpha, IL6, MIP-2, MMP-9, FDP, Substance P, iNOS in liver.

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 Anti-inflammatory and Other Biomarkers

With the continued treatment to the respective groups of 8th week of the experimental period, all the animals were sacrificed, liver were collected, homogenized and subjected for the estimation of vital biomarkers. The tissue from all the groups was stored at -20°C for further estimation. Alternatively, aliquot all the samples and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles, which may alter the level of biomarkers during final calculations.

Estimation of Anti-inflammatory and Other Biomarkers

The liver from all the groups was subjected for the estimation of level of vital functional anti-inflammatory biomarkers such as TNF alpha (CSB-E11987r), IL6 (CSB-E04640r), MIP-2 (CSB-E07419r), and other vital biomarkers in such as MMP-9 (CSB-E08008r), FDP (CSB-E07942r), Substance P (CSB-E08358r), iNOS (CSB-E08325r). All the biomarkers were estimated 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

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 1. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of TNF-α as 1914.43 ± 268.32 pg/mL, which was increased by 126.39% as compared with the normal control (G1, 845.64 ± 129.42 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) showed significant ($p \leq 0.001$) decreased TNF-α level by 36.49% *i.e.*, 1215.88 ± 140.70 pg/mL as compared to the G2 group. The level of TNF-α was significantly ($p \leq 0.001$) decreased by 46.94% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) group as compared to the disease control group (G2). Similarly, TNF-α level was decreased by 55.91% in the G6 group as compared to the untreated test formulation (G4). Inflammatory syndrome to systemic inflammatory response syndrome (SIRS) are associated with the multi-organ dysfunction syndrome (MODS). Tumor necrosis factor alpha (TNF-α) is proinflammatory cytokine that may link inflammation to

SIRS, that ultimately leads to MODS [30]. Therefore, in this experiment the Biofield Energy Treatment *per se* to the animals significantly reduced the level of TNF-α, which could be beneficial in the inflammatory disease conditions.

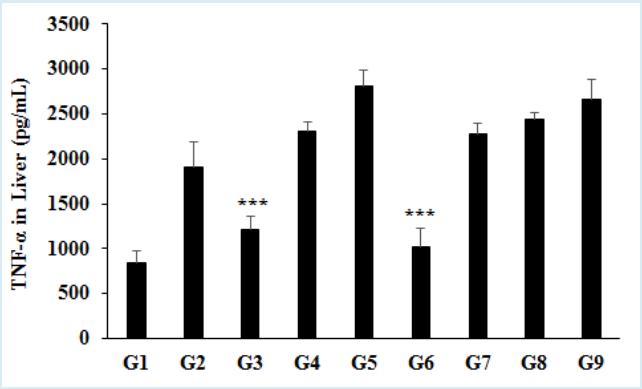

Figure 1: The effect of the test formulation on the level of liver tumour necrosis factor alpha (TNF-α) in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na); G3 as reference item (Cecal Slurry, LPS and *E. coli* + 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. G2.

Estimation of Interleukin-6 (IL-6)

The effect of the test formulation and Biofield Energy Treatment *per se* on the level of liver 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 0.67 ± 0.07 ng/mL, which was increased by 61.49% as compared with the normal control (G1, 0.42 ± 0.06 ng/mL). Further, the positive control (Dexamethasone) treatment (G3) showed decreased IL-6 level by 23.45% *i.e.*, 0.51 ± 0.04 ng/mL as compared to the G2 group. The level of IL-6 was significantly decreased by 15.30%, 51.44% ($p \leq 0.001$), 10.35%, 11.04%, and 16.35% in the G4 (Cecal Slurry, LPS and *E. coli* along with untreated 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) groups, 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). Similarly, IL-6 level was decreased by 42.92% and 1.68% in the G6 and G9 groups, respectively as compared to the untreated test formulation (G4). Based on the literature, it has been found that IL-6 is an early biomarker for sepsis. According to Mokart D (2005), the level of IL-6 was observed higher in the postoperative septic patients [31]. Combination of SIRS score and serum level of IL-6 can be early predictor of illness severity reported by Gregoric P, et al. [32]. 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 inflammatory conditions and simultaneously reduce the risks of inflammatory diseases.

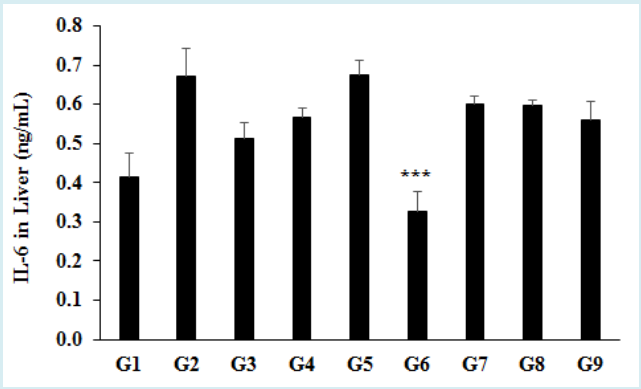

Figure 2: The expression of liver interleukin-6 (IL-6) after treatment with Biofield blessed proprietary test formulation and Biofield Blessing *per se* to Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na); G3 as 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* 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.* G2.

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

The level of liver macrophage inflammatory protein-2 (MIP-2) 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) showed value of MIP-2 as 608.67 ± 52.54 ng/mL, which was increased by 2.08% as compared with the normal control (G1, 596.24 ± 65.70 ng/mL). Further, the positive control (Dexamethasone) treatment (G3) showed the level of MIP-2 as 662.98 ± 61.67 ng/mL as compared to the G2 group. The level of MIP-2 was decreased by 21.52% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) groups as compared to the disease control group (G2). Similarly, MIP-2 level was significantly ($p \leq 0.001$) decreased by 31.54% in the G6 group with reference to untreated test formulation (G4) group. Based on the literature based study it has been reported that an increased plasma concentrations of proinflammatory cytokine like MIP-2, MCP-1, and eotaxin that leads to early deaths. These elevations occurred simultaneously for both the pro- and anti-inflammatory mediators [33]. Overall, here the Biofield Energy Treatment *per se* to the animals significantly reduced the level of MIP-2, which could be beneficial for the management of inflammatory disorders.

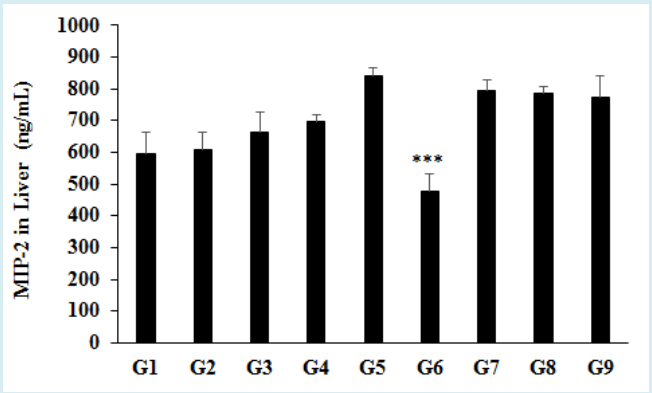

Figure 3: The expression of lungs macrophage inflammatory protein-2 (MIP-2) in Sprague Dawley rats after treatment with Biofield energized proprietary test formulation and animals *per se*. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% 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* 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.

Estimation of Matrix Metallopeptidase 9 (MMP-9)

Expression the level of liver matrix metallopeptidase 9 (MMP-9) in Sprague Dawley rats after administration of Biofield Treated/Blessed proprietary test formulation and Biofield Energy Healing/Blessing *per se*, and the results are graphically presented in the Figure 4.

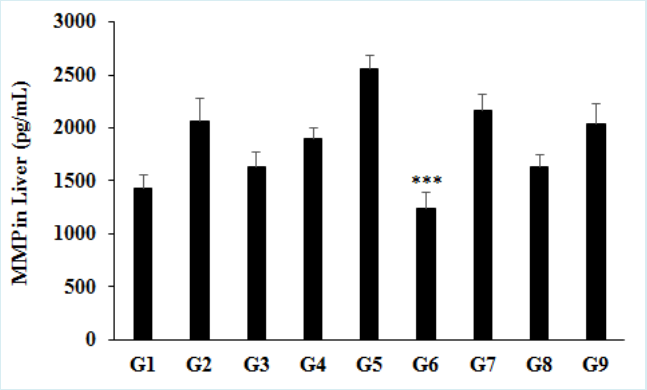

Figure 4: Expression the level of liver matrix metallopeptidase 9 (MMP-9) in Sprague Dawley rats after administration of Biofield Treated test formulation and Biofield Energy Healing *per se*. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% 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* 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.* G2.

The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of MMP-9 as 2061.25 ± 213.23 pg/mL, which was increased by 44.46% as compared

with the normal control (G1, 1426.91 ± 125.51 pg/mL). Further, the positive control (Dexamethasone) treatment (G3) group decreased MMP-9 level by 20.79% *i.e.*, 1632.71 ± 143.67 pg/mL as compared to the G2 group. The level of MMP-9 was significantly decreased by 7.62%, 40.13% ($p \leq 0.001$), 20.92%, and 1.33% in the G4 (Cecal Slurry, LPS and *E. coli* along with untreated test formulation); G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals 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 group (G2).

Besides, the level of MMP-9 was significantly reduced by 35.19% and 14.39% in the G6 and G8 groups, respectively with reference to untreated test formulation (G4) group. MMP-9 plays a vital roles in immune cell function and acts as modulators of inflammation. The expression of MMP-9 is upregulated during inflammatory conditions like arthritis, diabetes, and cancer [34,35]. In this study, 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.

Estimation of Fibrin Degradation Products (FDP)

Estimation the level of liver fibrin degradation products (FDP) in Sprague Dawley rats after administration of Biofield Treated the test formulation and Biofield Energy Healing *per se*, and the results are graphically shown in Figure 5. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of FDP as 8.32 ± 1.06 ng/mL, which was increased by 29.19% as compared with the normal control (G1, 6.44 ± 1.04 ng/mL). Further, the positive control (Dexamethasone) treatment (G3) showed the level of FDP as 9.58 ± 1.32 ng/mL. The level of FDP was significantly ($p \leq 0.001$) decreased by 38.22% and 50.53% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) group as compared to the disease control group (G2) and untreated test formulation group (G4), respectively. Sepsis is associated with systemic inflammatory responses and induction of intravascular fibrin formation. Based on one of the clinical trials observation, reported that patients with SIRS and associated with sepsis the level of FDP is too high in comparison with the healthy individuals [36]. Overall, here the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of FDP, which could be beneficial in the SIRS and sepsis patients.

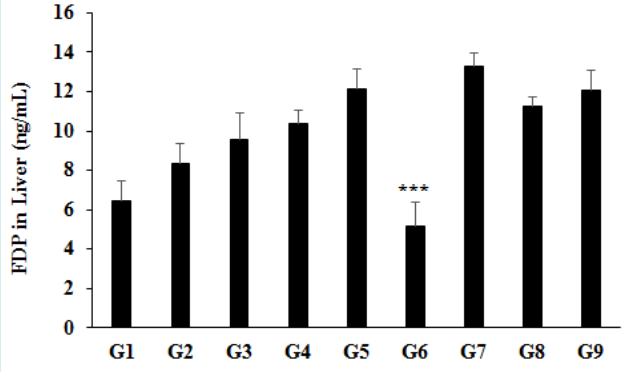

Figure 5: Estimation the level of liver fibrin degradation products (FDP) in Sprague Dawley rats after administration of Biofield Treated the test formulation and Biofield Energy Healing *per se*. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 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* 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.

Estimation of Substance P

The level of liver substance P was detected in all the experimental groups and the data are shown in Figure 6. The disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na) group (G2) showed value of substance P as 10.98 ± 0.86 ng/mL and which was increased by 23.09% as compared to the 8.92 ± 1.19 ng/mL, respectively. Further, the positive control (Dexamethasone) treatment (G3) showed decrease the level of substance P by 14.7% *i.e.*, 9.36 ± 0.67 ng/mL as compared to the G2 group. The level of substance P was significantly ($p \leq 0.001$) decreased by 36.96% and 38.20% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) as compared to the disease control group (G2) and untreated test formulation group (G4), respectively. According to Ang SF, et al. (2011), reported that the expression of substance P has increased in inflammation/septic condition through the activation of the

ERK-NF-κB pathway [37]. Overall, here the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* has significantly reduced the level of substance P, which could be beneficial for the management of systemic inflammation-related disorders.

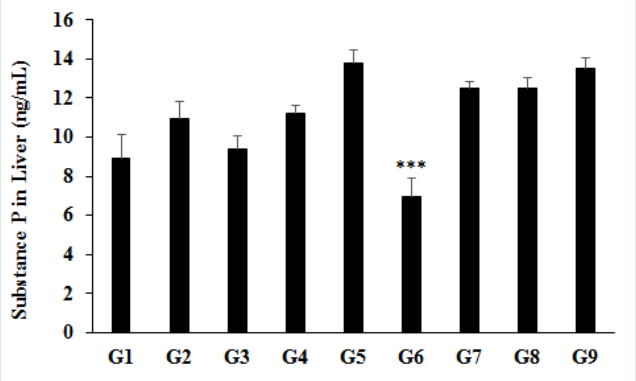

Figure 6: Expression of liver Substance P in Sprague Dawley rats after administration of Biofield Treated/ Blessed test formulation and Biofield Energy Healing/ Blessing *per se*. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na); G3 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* 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*. G2.

Estimation of Inducible Nitric Oxide Synthase (iNOS)

The level of liver inducible nitric oxide synthase (iNOS) 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 iNOS as 381.69 ± 42.83 IU/mL, which was increased by 22.01% as compared with the normal control (G1, 312.83 ± 43.21 IU/mL). Further, the positive control (Dexamethasone) treatment (G3) showed the level of iNOS was 419.9 ± 41.18 IU/mL. The level of iNOS was decreased by 19.19% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) as compared to the disease control group (G2). Similarly, iNOS level was significantly decreased by 35.41% ($p \leq 0.001$) and 5.46% in the G6 and G8 groups, respectively with reference to untreated test formulation (G4) group (Figure 7). Nitric oxide (NO) is the key endothelium-derived relaxing factor that maintain the vascular tone and reactivity. More generation of NO by the stimulation of iNOS have been proposed as a major mechanism of endothelial dysfunction, and that causes o abnormalities [38]. Besides, iNOS is expressed due to the effects of proinflammatory cytokines and can release more NO than other isoform of nitric oxide synthase enzymes [39]. Overall, here the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of iNOS, which could be beneficial for the management of inflammation-related disorders.

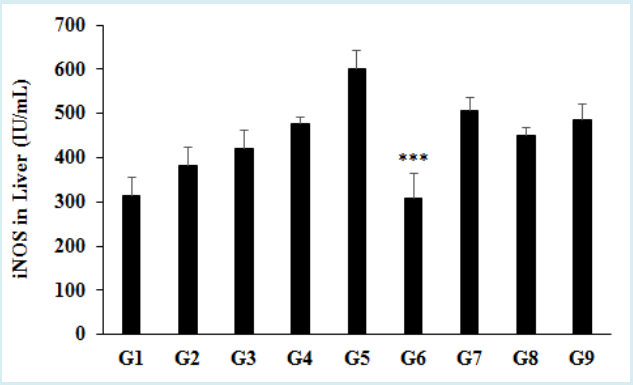

Figure 7: The effect of the test formulation on the level of liver Inducible Nitric Oxide Synthase (iNOS) in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (Cecal Slurry, LPS and *E. coli* + 0.5% CMC-Na); G3 as reference item (Cecal Slurry, LPS and *E. coli* + 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* 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.

The main objective was to investigate the anti-inflammatory potential of Mr. Trivedi's Biofield Energy/ Blessing (Prayer) on the novel Proprietary Test Formulation and *per se* to the animals on Cecal Slurry, LPS, and *E. coli*-induced systemic inflammatory response syndrome (SIRS) model in Sprague Dawley rats. As per study rational, authors included four preventive (day -15) treatment groups (G6, G7,

G8 and G9). The outcomes of this study showed the significant reduction of inflammatory biomarkers (TNF-α, IL-6, MIP-2, MMP-9) and others related biomarkers like FDP, substance P, and iNOS. Therefore, Biofield Treated Test formulation and animals treatment *per se* 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 TNF-α was significantly ($p \leq 0.001$) reduced by 46.94% and 55.91% in the G6 (Cecal Slurry, LPS and *E. coli* along with Biofield Energy Treatment *per se* to animals from day -15) group as compared to the disease control (G2) group and the untreated test formulation (G4), respectively. The expression of IL-6 was significantly ($p \leq 0.001$) reduced by 51.44% and 42.92% in the G6 group with reference to disease control (G2) group and untreated test formulation group, respectively. Moreover, MIP-2 level was significantly decreased by 21.52% and 31.54% ($p \leq 0.001$) in the G6 group with reference to G2 and G4 groups, respectively. The level of MMP-9 was significantly decreased by 40.13% ($p \leq 0.001$) and 20.92% in the G6 and G8 groups, respectively with reference to G2 group. FDP level was significantly ($p \leq 0.001$) decreased by 38.22% and 50.53% in the G6 group as compared to the G2 and G4 groups, respectively. Further, substance P was significantly ($p \leq 0.001$) decreased by 36.96% and 38.20% in the G6 group with reference to G2 and G4, groups, respectively. Besides, expression of iNOS was significantly ($p \leq 0.001$) decreased by 35.41% in the G6 group as compared to the G4 group. Altogether, 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 in Cecal Slurry, LPS and *E. coli*-induced systemic inflammatory response syndrome (SIRS) 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.

Acknowledgements

The authors are grateful to Dabur Research Foundation, Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for the assistance and support during the work.

References

1. Chakraborty RK, Burns B (2020) Systemic Inflammatory Response Syndrome. [Updated 2020 Apr 28]. StatPearls Publishing; Treasure Island (FL).

2. Balk RA (2014) Systemic inflammatory response syndrome (SIRS): Where did it come from and is it still relevant today?. Virulence 5(1): 20-26.

3. Comstedt P, Storgaard M, Lassen AT (2009) The systemic inflammatory response syndrome (SIRS) in acutely hospitalised medical patients: A cohort study. Scand J Trauma Resusc Emerg Med 17: 67.

4. Szekely Y, Arbel Y (2018) A review of interleukin-1 in heart disease: Where do we stand today? Cardiol Ther 7(1): 25-44.

5. Bishehsari F, Sharma A, Stello K, Toth C, Evans AC, et al. (2012) TNF-alpha gene (TNFA) variants increase risk for multi-organ dysfunction syndrome (MODS) in acute pancreatitis. Pancreatology 12(2): 113-118.

6. Qin CC, Liu YN, Hu Y, Yang Y, Chen Z, et al. (2017) Macrophage inflammatory protein-2 as mediator of inflammation in acute liver injury. World J Gastroenterol 23(17): 3043-3052.

7. Salvemini D, Riley DP (2000) Nonpeptidyl mimetics of superoxide dismutase in clinical therapies for diseases. Cell Mol Life Sci 57(11): 1489-1492.

8. Reshma VJ, Anwar AS, Mufeed A, Roshni A (2015) Estimation of plasma fibrinogen degradation products in oral submucous fibrosis: A clinico-pathological study. J Int Soc Prev Community Dent 5(4): 309-313.

9. Sternberg EM (2006) Neural regulation of innate immunity: A coordinated nonspecific host response to pathogens. Nat Rev Immunol 6: 318-328.

10. Tsukahara Y, Morisaki T, Horita Y, Torisu M, Tanaka M, et al. (1998) Expression of inducible nitric oxide synthase in circulating neutrophils of the systemic inflammatory response syndrome and septic patients. World J Surg 22: 771-777.

11. Rayman MP (2000) The importance of selenium to human health. Lancet 356(9225): 233-241.

12. Beard JL, Connor JR (2003) Iron status and neural functioning. Ann Rev Nutr 23: 41-58.

13. Peres FF, Lima AC, Hallak JEC, Crippa JA, Silva RH, et al. (2018) Cannabidiol as a promising strategy to treat and prevent movement disorders? Front Pharmacol 9: 482.

14. Nagarkatti P, Pandey R, Rieder SA, Hegde VL, Nagarkatti M (2009) Cannabinoids as novel anti-inflammatory drugs. Future Med Chem 1(7): 1333-1349.

15. Kang S, Min H (2012) Ginseng, the 'Immunity Boost': The effects of *Panax ginseng* on immune system. J Ginseng Res 36(4): 354-368.

16. Maizes V, Rakel D, Niemiec C (2009) Integrative medicine and patient-centered care. Explore (NY) 5(5): 277-289.

17. Bischof M, Del Giudice E (2013) Communication and the emergence of collective behavior in living organisms: a quantum approach. Mol Biol Int 2013: 987549.

18. Cassidy CM (2004) What does it mean to practice an energy medicine? J Altern Complement Med 10(1): 79-81.

19. Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 12: 1-23.

20. Wai FK (2005) National center for complementary and alternative medicine website. J Med Libr Assoc 93: 410-412.

21. Wisneski L, Anderson L (2009) The Scientific Basis of Integrative Medicine. Boca Raton, FL: CRC Press 205.

22. Trivedi MK, Branton A, Trivedi D, Jana S (2021) Isotopic abundance ratio analysis of consciousness energy healing treated folic acid. Food Nutr Current Res 4(2): 290-295.

23. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica* L.). Journal of Food and Nutrition Sciences 3: 245-250.

24. Trivedi MK, Jana S (2019) *In vitro* assessment of the biofield treated test item on cardiac function using rat cardiomyocytes cell line (H9c2) *via* multiparametric analysis. Journal of Hypertension and Cardiology 2(4): 1-12.

25. Trivedi MK, Branton A, Trivedi D, Jana S (2021) Effect of consciousness energy healing treatment on the metal profile and properties of tellurium. Eng Technol Open Acc 3(5): 555623.

26. Mahendra KT, Alice B, Dahryn T, Snehasis J (2021) Consciousness energy healing treatment impacted the isotopic abundance ratio of 6-Mercaptopurine (6-MP). Nov Appro Drug Des Dev 5(5): 555673.

27. Trivedi MK, Jana S (2021) Anti-aging activity of biofield energy treated novel proprietary test formulation by assessment of vital biomarkers in cerebrospinal fluid (CSF) in Sprague Dawley rats. On J Neur Br Disord 5(2).

28. Trivedi MK, Jana S (2021) Evaluation of biofield energy healing treatment based proprietary test formulation on gut health potential in colon cancer cell line (HT-29). J Pharmacol Clin Res 8(4): 555743.

29. Trivedi MK, Branton A, Trivedi D, Jana S (2020) The consciousness energy healing treatment and its impact on the isotopic abundance ratio analysis of flutamide. Drug Des Int Prop Int J 3(5): 2020.

30. Bishehsari F, Sharma A, Stello K, Toth C, Evans AC, et al. (2012) TNF-alpha gene (TNFA) variants increase risk for multi-organ dysfunction syndrome (MODS) in acute pancreatitis. Pancreatology 12(2): 113-118.

31. Mokart D, Merlin M, Sannini A, Brun JP, Delpero JR et al. (2005) Procalcitonin, interleukin 6 and systemic inflammatory response syndrome (SIRS): Early markers of postoperative sepsis after major surgery. Br J Anaesth 94(6): 767-773.

32. Gregoric P, Sijacki A, Stankovic S, Radenkovic D, Ivancevicet N, et al. (2010) SIRS score on admission and initial concentration of IL-6 as severe acute pancreatitis outcome predictors. Hepatogastroenterology 57(98): 349-353.

33. Osuchowski MF, Welch K, Siddiqui J, Remick DG (2006) Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. J Immunol 177(3): 1967-1974.

34. Halade GV, Jin YF, Lindsey ML (2013) Matrix metalloproteinase (MMP)-9: A proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. Pharmacol Ther 139(1): 32-40.

35. Lee AC, Trivedi MK, Branton A, Trivedi D, Nayak G, et al. (2018) The potential benefits of biofield energy treated vitamin D_3 on bone mineralization in human bone osteosarcoma cells (MG-63). International Journal of Nutrition and Food Sciences 7(1): 30-38.

36. Toh JM, Dror KG, Downey C, Abrams ST (2013) The clinical utility of fibrin-related biomarkers in sepsis.

Blood Coagul Fibrinolysis 24(8): 839-843.

37. Ang SF, Moochhala SM, MacAry PA, Bhatia M (2011) Hydrogen sulfide and neurogenic inflammation in polymicrobial sepsis: Involvement of substance P and ERK-NF-κB signalling. PLOS ONE 6(9): e24535.

38. Tang EH, Vanhoutte PM (2010) Endothelial dysfunction: A strategic target in the treatment of hypertension? Pflugers Arch 459(6): 995-1004.

39. Besedina A (2016) NO-synthase activity in patients with coronary heart disease associated with hypertension of different age groups. J Med Biochem 35(1): 43-49.