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Antioxidant Effects of the Biofield Energy Treated Novel Proprietary Test Formulation in Male Wistar Rats

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Abstract

To determine the antioxidant potential of the Biofield Energy (the Trivedi Effect®) Treated novel proprietary test formulation and Biofield Energy Treatment *per se* on male rats. The test formulation was distributed into two parts; one was denoted as the untreated formulation, while the other was treated with Biofield Energy by Mahendra Kumar Trivedi and denoted as Biofield Treated group. Besides, three group of animals were also received Biofield Energy Treatment under same conditions. The results showed that Malondialdehyde (MDA) was significantly reduced by 30.95%, 17.30%, and 45.76% ($p \leq 0.05$) in the Biofield Treatment *per se* to animals at day -15 (G6), Biofield Treated test formulation from day -15 (G7), and Biofield Treatment *per se* to animals with the untreated test formulation (G9) groups, respectively compared to the disease control (G2) group. Moreover, Myeloperoxidase (MPO) level was altered by 65.57%, 41.27%, 51.18%, and 49.29% in the G6, G7, Biofield Treatment *per se* to animals with Biofield Energy Treated test formulation from day -15 (G8), and G9, respectively compared to the G2. Superoxide dismutase (SOD) level was significantly ($p \leq 0.001$) increased by 81.47%, 95.87%, 74.66%, and 83.88% in the G6, G7, G8, and G9, respectively compared to the G2. Additionally, Catalase (CAT) activity was significantly increased by 31.69% and 12.28% in the G5 and G6, respectively compared to the G2. Further, glutathione (GSH) level was significantly increased by 12.54% in the G7, however; glutathione peroxidase (GPx) level was significantly ($p \leq 0.001$) increased by 125%, 116.86%, and 174.42% in the G7, G8 and G9, respectively with respect to the G2. The body weight, feed consumption, water intake, and relative organ weight results suggest that the Biofield Treated formulation did not show any signs of organ-related toxicity and it considered as safe compared to the normal control. Histopathological findings also supported that the Biofield Treatment group did not show any treatment-related changes in all the experimental animals as compared with the normal control. Overall, data suggests that Biofield Treatment *per se* (The Trivedi Effect®) and Biofield Treated test formulation possess significant antioxidant activity in order to improve and boost the immune system. Therefore, this therapy could be useful for the management of stress and various immune-related disorders like a plastic Anemia, Pernicious Anemia, Systemic Lupus Erythematosus, Myasthenia Gravis, Rheumatoid Arthritis, Addison Disease, Multiple Sclerosis, Graves ' disease, Reactive Arthritis, *etc.*

Keywords: Antioxidant; Biofield Energy Healing; LPO; MPO; SOD; Catalase; GSH; GPx; Histopathology

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Introduction

Oxidative stress is the primary cause for many diseases[1]. It has been well proven that reactive oxygen species (ROS) can directly causes oxidative injury to cells by damaging cell membrane, lipids, proteins, and nucleic acids in tissues[2]. The human has the excellent antioxidant defense system to protect the ROS. Decreased antioxidant system activities and increased ROS production leads to pathogenesis of many diseases like hypertension, atherosclerosis, diabetes, chronic renal disease, cancer, rheumatoid arthritis, ischemia / reperfusion, chronic adenotonsillitis, and aging[3,4]. Antioxidant activity is considered as one of the vital property of any formulation or nutraceuticals. However, the high concentration of free radicals is very much accountable for abundant inflammatory infections[5]. Myeloperoxidase (MPO) is a pro-oxidant with antimicrobial activity. By the utilization of H_2O_2 it produced Hypochloric acid (HClO) and other toxic substances in neutrophil phagolysosomes. It also causes neurodegenerative disorders and atherosclerosis[6,7]. The minerals based formulation is believed to improve the immune system by sustaining the body self-defense mechanism and re-establish the body's equilibrium. Literature suggests that most of the immunomodulatory formulation are based on medicinal plants, minerals, and organic matter[8]. Minerals and plant based product have reported with limited and low toxicity that make them ideal moieties for drug formulations[9]. The trace minerals like selenium, zinc, copper, magnesium, *etc*. have been reported for important role in immunomodulation[10]. Due to continued scientific research a new proprietary formulation was designed for antioxidant activity. The test formulation contained Nanocurcumin, Zinc chloride, Magnesium (II) Gluconate, Sodium selenate, Ascorbic acid (Vit-C), Cholecalciferol (Vit-D3), Iron (II) Sulfate, and Copper chloride.

It might be expected that all the constituents in the formulation may interact with co-ordinate fashion with the immune cells that can evoke an appropriate free radical scavenging response. All the constituents has been reported to have different biological activities such as antioxidant, anti-inflammatory, anti-viral, and immune modulating[11], and plays an important role in the treatment of inflammation and metabolic diseases[12].

Consciousness Energy Healing as a Complementary and Alternative Medicine (CAM) has been reported with an improved immune response with several benefits in various forms[13]. Researchers reported on the basis of reports and clinical trials, the importance of Biofield Energy Healing on immune system such as in case of improved immune function in cervical cancer patients after therapeutic touch[14] and massage therapy[15]. However, energy can exists in various forms that can be harnessed and transmit it into living and non-living things by the process of Biofield Energy Treatment. The Trivedi Effect® had been expansively reported with significant results in different scientific fields like cancer research[16,17], microbiology[18-21], genetics[22,23], pharmaceutical science[24-27], agricultural science[28-31], and materials science[32-35]. Thus, study has been designed to evaluate the impact of Biofield Treated formulation and Biofield Treatment *per se* antioxidant effect using various antioxidants like tissue Lipid Peroxidation (LPO), Myelo Peroxidase (MPO), Superoxide dismutase (SOD) and Catalase (CAT), Glutathione (GSH) and Glutathione Peroxidase (GPx), *etc*.

Material and Methods

Chemicals and Reagents

Cyclophosphamide and Carboxymethylcellulose sodium were purchased from Sigma Chemical Co. (St. Louis, MO). Nanocurcumin (purity 40%) was obtained from Sanat Products Ltd., India. Magnesium (II) gluconate hydrate and zinc chloride were procured from TCI, Japan; while sodium selenate was procured from Alfa Aesar, USA. Levamisole hydrochloride, ascorbic acid, cholecalciferol, and iron (II) sulfate were procured from Sigma, USA. Copper Chloride was purchased from VETEC (Sigma-Aldrich), USA.

Laboratory Animals: A total number of 72 healthy Wistar male rats (8 animals in each groups), weighing between 200-275 grams, were used in this experiment. Animals were kept under standard experimental conditions, with room temperature and relative humidity maintained at 22 ± 3°C and 30% to 70%, respectively. The animals were acclimatized prior to the experiment, and all were accessed once daily for clinical signs, behaviors, morbidity and mortality. The animal care was 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 was registered for experiment of animals. The animals were procured using Animal Ethics Committee approved protocol) and the husbandry conditions maintained as per CPCSEA recommendations.

Biofield Energy Treatment Strategy: The test formulation was divided into two parts. One part of the test formulation was treated with the Biofield Energy by renowned Biofield Energy Healer (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated formulation, while the second part of the test formulation did not receive any treatment and was defined as the untreated test formulation. The Biofield Energy Healing Treatment was provided by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi for ~3 minutes through the Healer's unique Energy Transmission process remotely to the test formulation present in the research laboratory of Dabur Research Foundation near New Delhi, India. Besides, three group of animals were also received Biofield Energy Treatment under laboratory conditions for ~3 minutes. Further, the control group was treated by a "sham" healer for comparative purposes. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples and animals were kept as per in-house conditions for experimental study.

Treatment Procedure: After one week of acclimatization, the animals were grouped (G) based on their body weight. G1 (normal control) received oral suspension of 0.5% carboxymethyl cellulose-sodium (CMC-Na) salt. All animals except G1 group received cyclophosphamide (at 25 mg / kg; *i.p.*) on day 9 and 16. G1, G2, and G6 groups were treated with 0.5% w/v CMC-Na in distilled water. G3 animals received reference item, levamisole hydrochloride at a dose of 50 mg / kg from day 1 to 22. G4 and G5 groups received the untreated and Biofield Energy Treated test formulation (at 624.115 mg / kg, *p.o.*). G6 and G8 groups

included Biofield Energy Treatment *per se* to the animals (-15 days). After 15 day pre-study period (G7 and G8 animals received the test formulation from day -15), while G9 group animals were treated with Biofield Energy Treatment *per se* along with the untreated test formulation for 22 days. On day 24th, 50% of animal and on day 25th remaining 50% of animal population from each group were sacrificed to collect various organs. A portion of liver sample was transferred into prescribed homogenizing buffer, homogenized to collect supernatant and stored in -80°C for the estimation of various anti-oxidant parameters like Lipid peroxidase (LPO), myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) using commercially available kit. All the organs were weighed and preserved in Normal Buffered Formalin (NBF) for histopathology (tissue health) examination.

Assessment of Antioxidant Activities

Tissue Lipid Peroxidation (LPO) in Liver Homogenate: Measurement of Thiobarbituric acid reactive species (TBARS) levels is considered as an index of Malondialdehyde (MDA) production[36]. This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with TBARS, a pink chromogen, which can be measured spectro photometrically at 532 nm, an MDA standard was used to construct a standard curve against which readings of the samples were plotted[37].

Tissue Myeloperoxidase (MPO) in Liver Homogenate: For MPO estimation, liver tissue (5% w/v) was homogenized in 0.5% hexa decyl tri methyl ammonium bromide (HTAB, Sigma-Aldrich, Co., St. Louis, MO, USA) with 50 mM potassium phosphate buffer, pH 6. The rest of the steps were performed as per in-house standard protocol. In addition, the homogenate was used for the estimation of Myeloperoxidase (MPO) using Elisa kit (Cat No: k11- 0575, Kinesisdx) through the colorimetric method as per manufacturer recommended standard procedure[38].

Superoxide dismutase (SOD) and Catalase (CAT): The liver homogenate was used as a matrix for the estimation of antioxidant enzymes by a colorimetric method with slight modification for SOD[39] and CAT[40]. Briefly, the formation of chromic acetate from dichromate and glacial acetic acid in the presence of hydrogen peroxide was measures calorimetrically at 570 nm. One enzyme unit was defined as the amount of enzyme which catalysed the oxidation of 1 µ M H_2O_2 per minute under assay conditions[41].

Glutathione (GSH) and Glutathione Peroxidase (GPx): For the estimation of GSH, the liver sample was used, which is based on the reduction of 5, 5 Di Thiobis (2-NitroBenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to the GSH concentration and its absorbance was measured at 405 nm by using a commercial kit (Item No: 703002, Cayman Chemicals)[42]. Liver tissues (GPx) enzyme activity was measured as IU / gm tissue by the reaction between glutathione remaining after the action of GPx and 5, 5-dithiobis-(2-nitrobenzoic acid) to form a complex that absorbs maximally at 412 nm. The sample absorbance was measured at 405 nm by using a commercial kit (Item No: 703102, Cayman Chemicals)[43].

Body Weight, Feed Consumption, and Water Intake: The feed intake, body weight, and water intake were recorded once daily before the test formulation administration throughout the experimental period. The daily feed intake was calculated from the difference between the weight of daily feed provide and the left-over feed[44,45].

Histopathology and Organ to Body Weight Ratio: Animals were euthanized by CO_2 asphyxiation as per in-house standard protocol. Selected organs were excised, weighed, and preserved for histopathological analysis as per standard protocol. The organ to body weight ratio of each rat was determined by comparing the absolute weight of each organ with the final body weight. Testis were fixed in modified Davidson fluid for 24 hour followed by 70% alcohol for 48 hours[46,47].

Statistical Analysis: The data were represented as Mean ± standard error of mean (SEM), N = 8. Student's t-test was used to compare two groups to judge the statistical significance. For multiple groups' comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis using Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Antioxidant Profile by ELISA Based Assay

Tissue Lipid Peroxidation (LPO) and Myeloperoxidase (MPO) in Liver Homogenate: The effect of the test formulation on lipid peroxidation is shown in Figure 1A and 1B. The lipid peroxidation end product is malondialdehyde (MDA). The level of MDA in the normal control (G1) group was 12.29 ± 0.75 µM and it was significantly increased by 46.70% in the disease control (G2; 18.03 ± 2.67 µM) group. The antioxidant enzymes such as lipid peroxidase (LPO), myeloperoxidase (MPO), and malondialdehyde (MDA) are excellent biomarkers for diagnosis of numerous immune-related diseases[48]. The positive control (levamisole) showed a significant ($p \leq 0.01$) reduction of MDA by 36.94% as compared to the disease control (G2). Besides, the untreated (G4) and Biofield Energy Treated (G5) test formulation showed 40.38% ($p \leq 0.05$) and 38.88% ($p \leq 0.05$) reduction of MDA level, respectively as compared to the G2 group. Moreover, the level of MDA was significantly reduced by 30.95%, 17.30%, 7.93%, and 45.76% ($p \leq 0.05$) in the Biofield Treatment *per se* to animals (-15 days) group (G6), Biofield Treated test formulation from day -15 (G7), Biofield Treatment *per se* to animals with Biofield Treated test formulation from day -15 (G8), and Biofield Treatment *per se* to animals with the untreated test formulation group (G9), respectively compared to the G2 group (Figure 1A). According to Lodi et al. 2011, a decreased LPO level clearly indicates the anti peroxidative activity[49]. Here, the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* to the animals showed significant inhibition of LPO in terms of the reduction of the MDA level, which might be due to free radical scavenging effect. Besides, the level of MPO after treatment with the test formulation is shown in Figure 1B. Further, level of MPO was significantly altered by 65.57%,

41.27%, 51.18%, 49.29% in the Biofield Treatment *per se* to animals (-15 days) group (G6), Biofield Treated test formulation from day -15 (G7), Biofield Treatment *per se* to animals with Biofield Treated test formulation from day -15 (G8), and Biofield Treatment *per se* to animals with the untreated test formulation group (G9), respectively compared to the G2 group (Figure 1B).

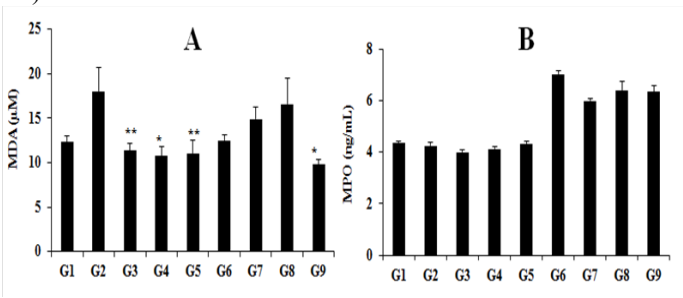

Figure 1: The effect of the test formulation on anti-oxidative markers A. lipid peroxidase (LPO), B. myeloperoxidase (MPO) after 23 consecutive days of treatment on various groups (G1 - G5) in male Wistar rats by oral route assessed in serum sample. G1: Normal control; G2: Disease control; G3: Levamisole hydrochloride; G4: Untreated test formulation; G5: Biofield Treated test formulation; G6: Biofield Treatment *per se* to animals (-15 days); G7: Biofield treated test formulation from day -15; G8: Biofield Treatment *per se* to animals with Biofield Treated test formulation from day -15; and, G9: Biofield Treatment *per se* to animals with untreated test formulation. *$p \leq 0.05$ and **$p \leq 0.01$ vs. G2.

Superoxide Dismutase (SOD) and Catalase (CAT) Activity in Liver Homogenate: The effect of Biofield Treated and untreated test formulation on the levels of various antioxidant enzymes such as Superoxide dismutase (SOD) and Catalase (CAT) in male Wistar rats is shown in Figure 2A and 2B. The antioxidant biomarkers such as SOD, and CAT were evaluated in liver samples. CAT is an essential enzyme for innate immunity. Further, CAT can correlate between the stress and immune response. It can maintain the oxidation-reduction (redox) balance by removing the hydrogen peroxide (H_2O_2) of immune system[50]. The level of SOD in the normal control group (G1) was 312.71 ± 28.41 U/mL and it was significantly reduced by 29.53% in the disease control group (G2). The positive control (levamisole) showed 52.40% increased of SOD level compared to the G2 group. Moreover, the level of SOD was significantly increased by 33.78%, 39.38%, 81.47%, ($p \leq 0.05$) 95.87%, ($p \leq 0.05$) 74.66%, and 83.88% ($p \leq 0.05$) in the untreated test formulation (G4) and Biofield Energy Treated test formulation (G5), Biofield Treatment *per se* to animals (-15 days) group (G6), Biofield Treated test formulation from day -15 (G7), Biofield Treatment *per se* to animals with Biofield Treated test formulation from day -15 (G8), and Biofield Treatment *per se* to animals with the untreated test formulation group (G9), respectively compared to the G2 group (Figure 2A).

Besides, the level of CAT in the normal control (G1) group was 16.79 ± 1.41 µmol / min / mL and it was significantly reduced by 23.88% in the disease control (G2; 12.78 ± 0.92 µmol / min/mL). The key role of antioxidant defense mechanism by CAT was due to the up-regulation of antimicrobial gene expression[51]. Moreover, the level of CAT was significantly increased by 16.35%, 31.69%, 12.28%, 3.44%, and 3.52% in the G4, G5, G6, G7, G8, and G9, respectively compared to the G2 group (Figure 2B). Due to macrophages activation there was a massive release of cytokines and enzymes that shape the inflammatory response that leads to increase the production of reactive oxygen species (ROS). Cu / Zn superoxide dismutase (SOD-1) is a vital enzyme responsible for the dismutation of superoxide radicals from cellular oxidative metabolism into hydrogen peroxide[52]. The Biofield Treated test formulation showed a significantly increased the level of SOD and CAT enzymes activities as compared to the disease control group. Based on literature[53], it is demonstrated that the Biofield Energy Treated test formulation might inhibits the release of various pro-inflammatory cytokines (TNF-α, VEGF) and metalloproteinase enzymes (MMP-2, MMP-9) and thus enhanced the immune activity. Overall, SOD and a CAT data suggest that the Biofield Treated test formulation could affect the immune response and pathologies.

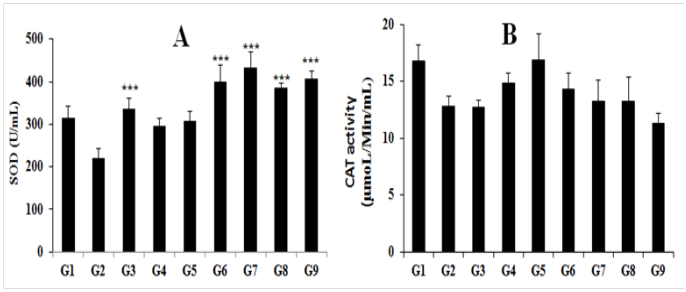

Figure 2: The effect of the Biofield Energy Treated test formulation on anti-oxidative markers A. Superoxide dismutase (SOD), B. Catalase (CAT) in male Wistar rats by oral route assessed in serum sample. ***$p \leq 0.001$ vs. G2.

GSH and GPx Activity: Antioxidant activity of the novel test formulation was studied using ELISA method by estimating various enzymes such as antioxidants *viz.* GPx and GSH. Liver homogenate of rat in various groups were used for the estimation of antioxidants enzymes and results are presented in Figure 3. The level of GSH in the normal control group was 191.10 ± 24.71 µM and it was significantly reduced by 20.92% in the disease control (G2) group (151.12 ± 18.81 µM). Moreover, the positive control, levamisole showed a significantly increased the GSH level by 30.79% compared to the disease control (G2) group. Further, the level of GSH was significantly increased by 5.49%, 8.64%, 12.54%, 1.26%, and 7.49% in the Biofield Energy Treated test formulation (G5), Biofield Treatment *per se* to animals (-15 days) group (G6), Biofield Treated test formulation from day -15 (G7), Biofield Treatment *per se* to animals with Biofield Treated test formulation from day -15 (G8), and Biofield Treatment *per se* to animals with the untreated test formulation group (G9), respectively compared to the untreated test formulation (G4) group (Figure 3A). Besides, the level of GPx in the normal control group (G1) was 4.24 ± 0.44 µM / min / mL and it was significantly reduced by 9.90% in the disease control group (G2). Moreover, levamisole showed 5.5% increment of GPx level compared to the G2 group. Additionally, GPx level was significantly increased by 7.56%, 21.51%, 125%, ($p \leq 0.05$) 116.86%, ($p \leq 0.05$) and 174.42% ($p \leq 0.05$) in the G5, G6, G7, G8 and G9 groups, respectively as compared to the diseases control group compared to the untreated test formulation (G4) group (Figure 3B). Antioxidant activity is considered as one of the vital property of any formulation or nutraceuticals. However, the high concentration of free radicals are very much account-

able for abundant inflammatory infections[54]. Overall, the experimental data suggested that the novel test formulation has the significant antioxidant activity, which might help to minimize the inflammatory responses against wide range of inflammatory disease conditions.

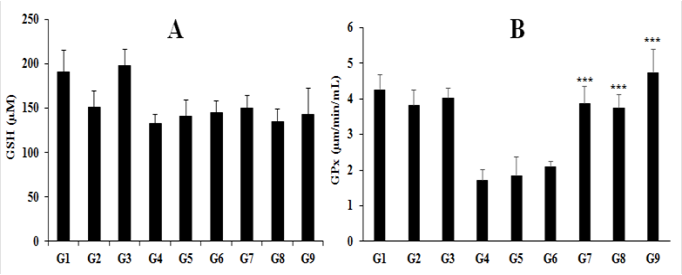

Figure 3: The effect of the Biofield Energy Treated test formulation (G5 to G9) except Biofield Energy Treatment to animals *per se* (G6) on anti-oxidative markers A. Glutathione (GSH), B. Glutathione peroxidase (GPx) in male SD rats by oral route assessed in serum sample. ***$p \leq 0.001$ vs. G2.

Body Weight, Feed Intake, Water Intake, and Relative Organ Weight Ratio: The Biofield Energy Treated test formulation possess consistent improvement of the body weight, feed intake and water intake. The absolute weight of various selective vital organs was recorded. From this, the relative organ weight ratio (as percentage) was calculated in all the groups and the data is presented in Table 1. Thus, the results suggest that the Biofield Energy Treated test formulation was consider being safe. The organ to body weight ratio is use as an indicator for the identification of swelling, atrophy or hypertrophy[55].

Macro and Microscopic Examination of Tissues: The effect of the Biofield Energy Treatment on test formulation on histopathological findings in male Wistar rats is shown in the Figure 4. Histopathological examination data did not show any drastic cellular changes that might be due to the toxic effect of the Biofield Energy Treated test formulation or *per se* treatment to animals directly. Decreased cellularity in white pulp and cortex and diffuse in spleen and thymus were observed in few animals in all the treatment groups (Figure 4).

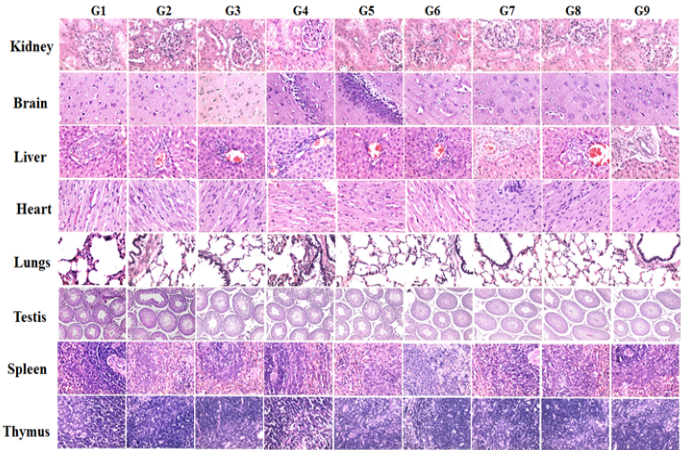

Figure 4: Histopathological photomicrograph of major organs tested after the effect of the Biofield Energy Treated test formulation (G5 to G9) except Biofield Energy Treatment to animals *per se* (G6) for consecutive 22 days in male Wistar rats. All the tissues were sectioned transversely and stained with hematoxylin (H) and eosin (E).

The Biofield Energy Treated novel proprietary test formulation have significant effect to improve antioxidant and overall health. Apart from the overall effect of the test formulation the Biofield Energy Treatment *per se* to the animals was also significantly improve the antioxidant activity. Overall, the Biofield Energy Treated test formulation might be considered as a safe supplementary therapy for immune modulation.

Conclusions

Based on the study results it was found that the end product of lipid peroxide i.e., malondialdehyde (MDA) was significantly reduced by 30.95 %, 17.30 %, and 45.76 % in the Biofield Treatment *per se* to animals (-15 days) group (G6), Biofield Treated test formulation from day -15 (G7), and Biofield Treatment *per se* to animals with the untreated test formulation group (G9), respectively compared to the disease control (G2) group. Other oxidative parameter like the level of Myeloperoxidase (MPO) was altered by 65.57 %, 41.27 %, 51.18 %, and 49.29 % in the G6, G7, G8, and G9, respectively compared to the G2 group. Antioxidant enzyme like superoxide dismutase (SOD) was significantly increased by 33.78 %, 39.38 %, 81.47 %, 95.87 %,

Table 1: The effect of the Biofield Energy Treated test formulation (G5 to G9) except Biofield Energy Treatment to animals *per se* (G6) on the relative organ weight ratio (percentage) of various vital organs in male Wistar rats.

Relative Organ Wt. (%)	G1	G2	G3	G4	G5	G6	G7	G8	G9
Liver	3.20 ± 0.09	3.38 ± 0.18	3.78 ± 0.17	3.58 ± 0.18	3.56 ± 0.18	3.41 ± 0.13	3.31 ± 0.08	3.55 ± 0.12	3.50 ± 0.09
Lungs	0.42 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.46 ± 0.03	0.45 ± 0.01	0.46 ± 0.03	0.43 ± 0.02	0.46 ± 0.02	0.44 ± 0.02
Kidneys	0.79 ± 0.04	0.85 ± 0.05	0.86 ± 0.06	0.83 ± 0.03	0.85 ± 0.04	0.80 ± 0.03	0.82 ± 0.04	0.81 ± 0.02	0.86 ± 0.03
Brain	0.61 ± 0.02	0.68 ± 0.02	0.68 ± 0.02	0.64 ± 0.02	0.59 ± 0.04	0.60 ± 0.04	0.61 ± 0.03	0.60 ± 0.01	0.64 ± 0.02
Heart	0.30 ± 0.01	0.33 ± 0.02	0.29 ± 0.01	0.31 ± 0.01	0.34 ± 0.02	0.36 ± 0.02	0.32 ± 0.02	0.32 ± 0.01	0.33 ± 0.01
Spleen	0.20 ± 0.01	0.16 ± 0.01	0.15 ± 0.00	0.18 ± 0.02	0.19 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
Small intestine	2.34 ± 0.20	2.14 ± 0.15	2.63 ± 0.14	2.48 ± 0.10	2.62 ± 0.23	2.29 ± 0.06	2.34 ± 0.09	2.49 ± 0.08	2.29 ± 0.10
Testis	1.05 ± 0.05	1.14 ± 0.04	1.18 ± 0.04	1.08 ± 0.03	1.10 ± 0.03	1.08 ± 0.03	1.10 ± 0.03	1.08 ± 0.02	1.07 ± 0.12
Prostate	0.22 ± 0.02	0.22 ± 0.02	0.17 ± 0.02	0.21 ± 0.02	0.19 ± 0.02	0.23 ± 0.02	0.21 ± 0.02	0.17 ± 0.02	0.19 ± 0.02
Epididymis	0.40 ± 0.02	0.45 ± 0.02	0.41 ± 0.02	0.39 ± 0.02	0.41 ± 0.01	0.44 ± 0.02	0.43 ± 0.01	0.41 ± 0.02	0.48 ± 0.02

74.66%, and 83.88% in the untreated test formulation (G4) and Biofield Energy Treated test formulation (G5), G6, G7, G8, and G9, respectively compared to the G2 group. Other Antioxidative enzyme like Catalase (CAT) activity was significantly increased by 16.35%, 31.69%, and 12.28% in the G4, G5, and G6, respectively compared to the G2 group. Further, GSH level was significantly increased by 12.54% in the G7 group however, GPx level was increased by 21.51%, 125%, 116.86%, and 174.42% in the G6, G7, G8 and G9 groups, respectively with respect to the G2 group. Further, physical parameters like body weight, feed consumption, water intake, and histopathological analysis did not show any abnormality compared to the normal control group. In conclusion, The Trivedi Effect®- Energy of Consciousness Healing Treated novel test formulation has enhanced the antioxidant response compared with the untreated test formulation, which can be used to fight against infectious diseases. Therefore, the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* may act as an effective antioxidant product. It can be used for various autoimmune disorders *viz.* Myasthenia Gravis, Aplastic Anemia, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Addison Disease, Reactive Arthritis, Multiple Sclerosis, Pernicious Anemia, Graves' Disease, Psoriasis, Type 1 Diabetes, Vitiligo, and Alopecia Areata, as well as inflammatory disorders *viz.* Crohn's Disease, Vasculitis, Ulcerative Colitis, Irritable Bowel Syndrome (IBS), Dermatitis, Asthma, Diverticulitis, Alzheimer's Disease, Atherosclerosis, Parkinson's Disease, and Hepatitis.

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