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Behavioural Parameters Analysis in Unpredictable Chronic Stress (UCS) Induced Animal Model after Treatment with the Biofield Energy Healing Based Test Formulation

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Abstract

The aim of the present study was to study the behavioural parameters in unpredictable chronic stress (UCS) of Consciousness Energy Healing Treatment (The Trivedi Effect®) based novel test formulation in male *Sprague Dawley* (SD) rat model. A test formulation composition included minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, alpha tocopherol, 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 untreated test formulation, while the other portion of test formulation and the animals received Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi. The experimental results showed Y maze test data showed time in start arm was significantly decreased by 76.92%, 34.33%, 24.63%, 55.52%, and 74.93% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. However, time in the novel arm was increased by 110.02%, 59.13%, 36.54%, 130.15%, and 97.61% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G4. Y maze test showed that entry in start arm was increased by 141.67% and 91.67% in the G6 and G7 group, respectively as compared with the G4. In addition, entry in explored arm was increased by 153.85% and 61.54% in the G6 and G7 groups, respectively as compared with the G4 group.

Morris water Maze results showed that maximum speed in zone was increased by 64.34%, 71.95%, 20.84%, 79.28%, and 109.49% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2. Similarly, the resting time in zone was significantly decreased by 86.27%, 82.74%, 77.89%, 53.86%, and 80.28% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2. Latency in target zone record showed that 87.74%, 87.94%, 66.89%, 87.59%, and 81.88% decreased values in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2, while entry in target zone was significantly increased by 215%, 110%, 190%, 125%, and 115% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2. Forced swim test data showed that number of climbing data suggested that G5, G6, G7, G8, and G9 groups showed an increased value by 101.34%, 146.04%, 93.04%, 141.30% and 125.39% respectively, as compared with the disease control G2 group, while immobility time (sec.) data showed that G5, G6, G7, G8, and G9 groups showed a decreased time by 51.58%, 70.95%, 37.05%, 73.35%, and 58.11% respectively, as compared with the disease control G2 group. Similarly, swim time data showed increased values by 21.69%, 29.83%, 15.58%, 30.84%, and 24.43% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group. However, total number of square crossed was increased by 62.18%, 94.55%, 26.55%, 25.80%, and

26.55% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group. Similarly, entries in the center zone was also significantly increased by 233.33%, 600%, 366.67%, 34.11%, and 200% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group.

Elevated Plus Maze results showed that time spent into the open arm was significantly increased by 2458%, 1460%, 1750%, 1079%, and 969% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. In addition, the entries in open arm behaviour was reported and found to be increased by 375%, 275%, 400%, 243%, and 200% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. On the other hand, sucrose preference test was significant increased by 37.09%, 35.63%, and 26.15% in the G5, G6, and G7 groups respectively, as compared with the G2. Overall, the data suggested a significant effect of Biofield Energy *per se* along with preventive measure on the animal with respect to various spatial learning and memory disorders. The results also showed the significant improvement of spontaneous alternation and a recognition memory test response that helps towards various diseases, allergies, lethargic conditions, energy booster action along with its associated complications/ symptoms can be preventive using Biofield Energy Treatment *per se* and/or Biofield Energy Treated Test formulation groups.

Keywords: Biofield Energy Treatment; Y maze test; Morris water Maze; Latency Time; The Trivedi Effect®; Unpredictable Chronic Stress

Abbreviations: UCS: Unpredictable Chronic Stress; SD: Sprague Dawley; EPM: Elevated Plus Maze; FST: Forced Swim Test; CAM: Complementary and Alternative Medicine; NCCAM: National Center for Complementary/Alternative Medicine; NCCIH: National Center of Complementary and Integrative Health; MWMT: Morris Water Maze Test; SPT: Sucrose Preference Test; ANOVA: Analysis of Variance.

Introduction

Stress is considered among the leading forms of psychiatric illness, which acts as a precipitating factor for anxiety-related disorders in the modern world. Therefore, there were various rodent-based behavioural models developed and the tests are nowadays, widely used to understand the mechanisms as well as to identify the new treatments for anxiety-related disorders [1]. Such models that helps in exploring the common behaviour and to investigate the presence of any stress include elevated plus maze, open-field activity, Y-maze test, forced swim test, sucrose preference test and Morris water maze test, etc [2]. The elevated plus maze (EPM) test involves the maze that consists of two opposing enclosed arms as well as two non-enclosed (open) arms, which makes the shape of a "plus sign", which is further elevated several feet above the ground. The results were analyzed from the latency to enter; time spent within; and number of entries into each arm type by the rats. Furthermore, such parameters helps in determining the proxies of anxiety levels, as the lower anxiety level could be interpreted by the animal spending greater amounts of time in the open arms [3,4].

Similarly, the assessment of anxiety and locomotor activity could be done by using the open field test that involves a wall-enclosed area, in the form of open field maze, which is of sufficient height to prevent the subject from escaping. In this test, the anxiety levels of animals could be inferred by their latency to enter as well as the time spent in the centre of the arena, where they were considered to be hypothetically most vulnerable to predators) [5,6]. The Y maze technique helps in studying the spatial learning and memory in rodents, and therefore, assessing the behavioural task in the preclinical research. It involves spontaneous alternation test and recognition memory test.; among which, the Y Maze spontaneous alternation test is based on the animals' natural curiosity to explore something. In this, the animal tries to explore a new arm of the maze rather than returning to the previously visited one. This task involves the use of many parts of the brain, such as, the hippocampus, basal forebrain, septum, and prefrontal cortex. The other Y maze test is the recognition memory test in which, the animal's preference to spend time in a novel or known area is analyzed. The test involves blocking the one arm of the Y-Maze and thereby allowing the animal to explore the other two arms. Therefore, it helps in testing the animal's memory function when the animal is returned to the maze with all arms open; and also helps in monitoring their tendency to spend time in the new arm or the known arms [7-9].

In the same way, the forced swim test (FST) is used for assessing the impact of various neurobiological and behavioural manipulations in basic and preclinical research. In this, the animal is placed in a container filled with water from which it cannot escape. Hence, the animal first tries to escape but eventually will exhibit immobility. Later on, it may start floating due to absence of any other movement that may help in keeping its nose above water [10,11]. The sucrose preference test helps in analysing the sensitivity of animals to reward in which, the animals have access to water without as well as with different concentrations of sucrose. The preference rate of animal is then analysed and further

used to assess the level of depression; as, the reduced interest in the reward might be caused by chronic stress, and may represent their depressive behaviour [12,13]. Besides, the Morris water maze test is used to study the spatial memory and learning behaviour of rodents. In this test, the animals are placed in a pool of water that is colored opaque, and they need to swim to a hidden escape platform. Since, they are in opaque water, it hinder their visibility to see the platform; therefore, in absence of sight and scent to find the escape route, they must rely on external/extra-maze cues [14,15].

In this study, a novel test formulation was designed to analyze any change in the behavioral parameters of animal, that include the combination of different minerals such as, selenium, iron, zinc, copper, calcium, and magnesium; vitamins such as, ascorbic acid, cyanocobalamin, pyridoxine HCl, cholecalciferol, and alpha tocopherol; cannabidiol isolate; and panax ginseng extract. All the ingredients used in the test formulation have been reported by various scientists for their significant physiological role [16-21]. Besides, the novel formulation and the animals *per se* were studied for behavioral studies in male *Sprague Dawley* rats using standard assays after treated with Biofield Energy Healing Treatment by a renowned Biofield Energy Healer. The Biofield Energy healing is considered as a novel approach, which was reported to have significant outcomes in the treatment of various disorders, without any adverse effects in comparison to the conventional medicine; and therefore, accepted worldwide by more than 80% of the US population in the form of Complementary and Alternative Medicine (CAM) treatment [22-24]. It is also recommended by the National Center for Complementary/Alternative Medicine (NCCAM) due to its more advantages over the current preferred treatment approach [25].

The other CAM therapies along with the Biofield Energy Healing, which are recognized and recommended by the National Center of Complementary and Integrative Health (NCCIH), are deep breathing, Tai Chi, yoga, therapeutic touch, Johrei, Reiki, polarity therapy, chiropractic/osteopathic manipulation, meditation, massage, homeopathy, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems, etc. [26,27]. The beneficial impact of the Trivedi Effect®-Consciousness Energy Healing Treatment has been scientifically studies in various fields such as bioavailability studies [28,29], metal science [30,31], agriculture science [32], biotechnology [33], microbiology [34,35], nutraceuticals [36], skin health [37,38], cancer research [39], bone health [40,41], and overall human health and wellness. The present study evaluated the animal behavioral parameters using Unpredictable Chronic Stress (UCS) induced, which was treated with Biofield Energy Treatment by a renowned Biofield Energy Healer.

Material and Methods

Chemicals and Reagents

Pyridoxine hydrochloride (vitamin B_6), calcitriol, zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, provit A) were purchased from TCI, Japan. Copper chloride, cyanocobalamin (vitamin B_{12}), calcium chloride, vitamin E (Alpha-Tocopherol), cholecalciferol (vitamin D_3), iron (II) sulfate, and sodium carboxymethyl cellulose (Na-CMC) were procured from Sigma-Aldrich, USA. Ascorbic acid (vitamin C) and sodium selenate were obtained from Alfa Aesar, India. Cannabidiol isolate and *panax ginseng* extract were obtained from Panacea Phytoextracts, India and Standard Hemp Company, USA, respectively. Imipramine Hydrochloride was purchased from Sigma, USA.

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 6 animals of each group. They were kept individually in sterilized polypropylene cages with stainless steel top grill having provision for holding pellet feed and drinking water bottle fitted with stainless steel sipper tube. The animals were maintained as per standard protocol throughout the experiment.

Consciousness Energy Healing Strategies

The novel test formulation was consisted of zinc chloride, iron (ii) sulfate, copper chloride, vitamin B_6, vitamin B_{12}, vitamin D_3, sodium selenate, calcium chloride, ascorbic acid, vitamin E, beta carotene, panax ginseng extract, cannabidiol and magnesium (II) gluconate. Each ingredient of the novel test formulation was divided into two parts. The test formulation was divided into two parts, one part of the test compound was not received 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 (n=10/per group) also received Biofield Energy Healing Treatment (known as the Trivedi Effect®) by Mahendra Kumar Trivedi under similar laboratory conditions for ~3 minutes. The test formulation were located in the research laboratory of Dabur Research Foundation, New Delhi, India. The energy transmission was done without touching the samples or animals. After that, the Biofield Energy Treated samples was kept in the similar sealed condition and used as per the study plan. In the same manner, the control test formulation

group was subjected to "sham" healer for 3 minutes energy treatment, under the same laboratory conditions. The sham healer not has any knowledge about the Biofield Energy Treatment. The Biofield Energy Treated animals were also taken back to experimental room for further proceedings.

Detailed 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; G2 as disease control (UCS: Unpredictable Chronic Stress with 0.5% CMC); G3 as reference item (UCS along with imipramine hydrochloride, 30 mg/kg); G4 includes UCS along with untreated test formulation; G5 as UCS along with Biofield Energy Treated test formulation); G6 group includes UCS along with Biofield Energy Treatment *per se* to animals from day -15; G7 as UCS along with Biofield Energy Treated test formulation from day -15; G8 group includes UCS along with Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9 group denoted UCS along with Biofield Energy Treatment *per se* animals plus untreated test formulation. G1 and G2 animals were treated orally with 0.5% w/v CMC-Na in distilled water for 8 weeks (From day 1 to 56).

Group G3 animal was treated orally with reference item, imipramine hydrochloride at a dose of 30 mg/kg body weight for 8 weeks. The freshly prepared suspensions of untreated test formulation and Biofield Energy Treated Proprietary Product was administered orally to the G4 and G5 group animals, respectively, at a dose of 990.56 mg/kg $BW^{-1}{\cdot}d^{-1}$ for 8 weeks. G6 group was not to be dosed with the test formulation. In addition; G7 and G8 group were dosed similar to the G4 and G5 dosing regimen, but from the day of Biofield Energy Treatment (*i.e.* from day-15 to day 56). G9 group, Biofield Energy Treated *per se* animal was treated with untreated test formulation for 8 weeks. Body weight and clinical signs were taken daily throughout the experimental period. All the animals except G1 group received stress induced procedures such as stress procedures like sound stress, tilted cages and crowd stress, cold and warm water swim stress, food and water deprivation, stress due to change in the light and dark cycle were undergo seven different types of unpredictable stress procedures after scheduled dosing daily at specified interval to the end of the experiment for 8 weeks after the initiation of stress, which vary every week interval *i.e.* shuffling of stress type. At the end of (8 week) experimental period, all the animals were individually subjected for various behavioral parameters.

Estimation of Behavioural parameter using Y-Maze Test

Impairment of spatial memory was evaluated using a Y-maze test paradigm. The Y-maze consisted of three equal-dimension arms with an angle of 120°. The arm closest to the experimenter was defined as the start arm in which rat was placed at the start of each trial. During trial 1 (5 min), the entrance to the novel arm was closed, limiting the animal to exploration of the start arm and the open arm. After a 30 min, animal was allowed to explore all three arms by having the entrance to the novel arm open. Exploratory behaviour was assessed for 5 min. In week 8, all the animals were individually subjected to Y-Maze test for 5 minutes. Video of Y-Maze test was recorded and analysed using SMART software to calculate the following parameters in case of novelty test includes estimation of time spent in each arm and number of entries made in to each arm, while spontaneous alteration (i.e. the successive entry of the rat into the three arms in overlapping triplet sets) includes percentage alteration behaviour that was calculated using equation:-

= [Successive triplet sets / Total number of arm entries - 2] X 100 (Successive triplet set: Entries into three different arms consecutively)

Estimation of Behavioural parameter using Morris Water Maze Test (MWMT)

All the animals were individually subjected to MWMT at the end of the experimental period in week-8. Videography of MWMT was recorded to analyse using SMART® software for following parameters such as total distance, resting time in zone, latency to target, maximum speed n in zone, and entries in zone among all the groups. The data was compared with respect to the positive control group and among the experimental test groups.

Estimation of Behavioural parameter using Forced Swimming Test

All the animals were individually subjected to swim in a cylinder (40 cm high, 18 cm in diameter) filled with water (25°C) up to height of 34 cm. Further, the video was recorded for 5 minutes to calculate the following parameters such as immobility time (in seconds), swim time (in seconds), and number of climbing by each animals among all the test groups.

Estimation of Behavioural parameter using Open Field Test

All the animals, individually subjected to open field test for 5 minutes. However, video was also recorded to calculate the following parameters such as total number of squares crossed, number of entries in centre squares, number of entries in side corners, defecation, and urination during

the experimental phase in all the animals. Further, the data was screened for the effect of test formulation which was compared with the positive control and untreated test formulation group.

Estimation of Behavioural parameter using Elevated Plus Maze (EPM)

Animals were placed at the centre of the EPM apparatus, facing one of the enclosed arms during a 5-min test period. Further, the video of EPM test was recorded to and analyzed using SMART® software. Following parameters were observed such as time spent in closed arm, and the time spent in open arm.

Estimation of Sucrose Preference Test (SPT)

All the animals were trained for 48 hour to adapt to 1% sucrose solution (*w/v*) at the beginning of the experiment in which two bottles with 1% sucrose solution were placed in each cage. After adaptation, the rats were deprived of water for 4 hour and then underwent the SPT, in which the rats were housed in individual cages for 1 hour and exposed to two identical bottles, one filled with 1% sucrose solution and the other filled with water. At the end of the 1 hour test, sucrose and water consumption was measured. Sucrose preference (%) was calculated using following equation-
Sucrose preference (%) = sucrose consumption/ (sucrose consumption + water consumption)

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

Effect of test formulation for behaviour parameters using Y maze test

Time spent in each arm was reported in the animals treated with UCS (G2), which showed more time spent in start arm, (124.72 ± 16.79) suggested impaired novelty-seeking behaviour and spatial memory as compared to the normal control (G1, 85.20 ± 8.73). However, marginal change was observed in imipramine treated (G3, 109.25 ± 12.87) group. The untreated test formulation to the untreated rats (G4) and Biofield Energy Treated test formulation (G5) groups showed a significant decrease (*p<0.01, p<0.001*) in time spent in start arm (*i.e.,* 46.37 ± 18.29 and 28.66 ± 10.78 respectively) as compared to the UCS (G2). Biofield Energy Treated animals (G6) and 15 days pre-treatment of Biofield Energy Treated test formulation (G7) showed a decreased (81.91 ± 13.01 and 94.00 ± 16.90) time spent as compared to the UCS (G2). 15 days pre-treatment of Biofield Energy Treated test formulation to the Biofield Energy Treated rats (G8) and the untreated test formulation to the Biofield Energy Treated rats (G9) showed a significant decrease *(p<0.05, p<0.001*) in the time spent in start arm, (55.47 ± 22.83 and 31.27 ± 11.71) as compared to the UCS (G2). However, animals with UCS (G2) showed a significant decrease ($p<0.05$) in time spent in novel arm (47.16 ± 9.62) as compared to the normal control (G1, 93.50 ± 17.04). However, the other experimental groups treated with reference compound, different treatment combination showed increased time spent in novel arm as compared with the G2.

Overall, time in start arm was significantly decreased by 76.92%, 34.33%, 24.63%, 55.52%, and 74.93% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. Similarly, time spent in explored arm was significantly decreased by 20.61%, 37.06%, 36.38%, 30.56%, and 22.17% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. However, 83.23%, 38.84%, 19.12%, 100.80%, and 72.40% increased time in the novel arm was reported in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. Similarly, time in the novel arm was increased by 110.02%, 59.13%, 36.54%, 130.15%, and 97.61% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G4.

Similarly, the number of entries into each arm data suggested that animals treated with the UCS (G2) showed a marginal decrease in number of entries in start arm, (2.13 ± 0.48) as compared to the normal control (G1, 2.63 ± 0.63). Imipramine treated (G3) group showed a significant decrease ($p<0.05$) in number of entries in start arm (0.88 ± 0.23) as compared to the UCS (G2). Whereas, the other treated groups showed marginal changes except untreated test formulation to the Biofield Energy Treated rats (G9) group showed a significant decrease ($p<0.05$) in entries in start arm, (0.75 ± 0.41) as compared to the UCS (G2). However, animals treated with UCS (G2) showed a significant decrease ($p<0.05$) in number of entries in novel arm, (2.75 ± 0.73) as compared to the normal control (G1, 6.25 ± 0.98). However marginal increase was observed in imipramine treated (G3) group (3.25 ± 0.73). Biofield Energy Treated animals (G6) group showed a significant increase ($p<0.05$) in the number of entries in novel arm, (5.00 ± 0.50) as compared to the UCS (G2). However, no increase was observed in number of entries in the novel arm among all the treated groups. Entry in start arm was significantly increased by 70.59% and 35.29% in the G6 and G7 groups, as compared with the G2, while entry in start arm was increased by 141.67% and 91.67% in the G6 and G7 group, respectively as compared with the G4. Besides,

entry in explored arm was increased by 153.85% and 61.54% in the G6 and G7 groups, respectively as compared with the G4 group. Y maze test is one of the standard tests for the estimation of spatial working and reference memory [42]. Novelty test was used to study the time spent in each arm and number of entries made into each arm.

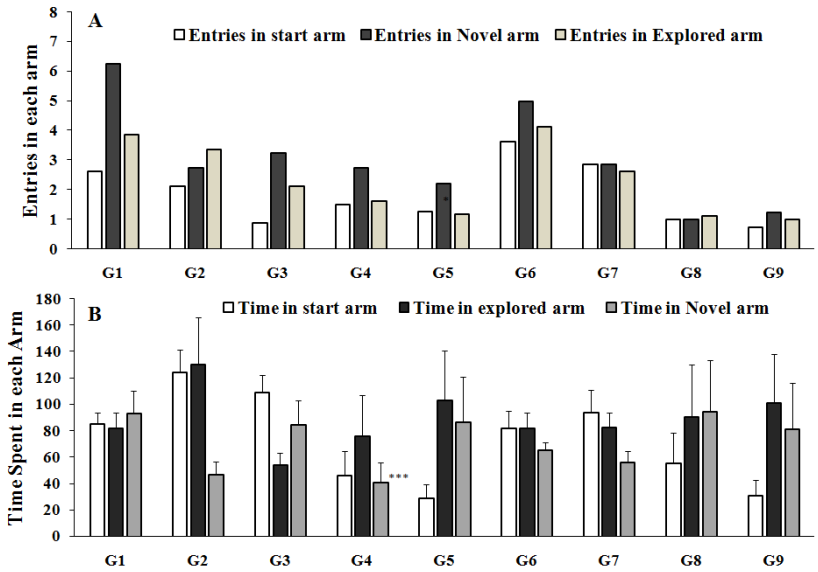

Figure 1: Effect of the test formulation evaluation of impairment of spatial memory using a Y-maze test in various test groups from G1 to G9 in male Sprague Dawley rats. (A) Entry in each arm, and (B) Time spent in each arm. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Effect of test formulation for behaviour parameters using Morris water Maze test

Morris water maze was performed for spatial memory and learning, which results on the basis of distal cues in order to navigate from start locations around the perimeter of an open swimming arena, which would help to locate a submerged escape platform. A target zone was fixed, all the animals were allowed to swim in the Morris water maze tank and check the different parameter of animals to reach the target zone [43]. Morris water Maze results were compiled and represented graphically (Figure 2). The animals were treated with UCS (G2) showed a significant decreased ($p<0.01$) value in maximum speed in zone (21.25 ± 2.14) and significant increase ($p<0.01$) in the resting time in zone (3.25 ± 0.78) as compared to the normal control (G1) (63.17 ± 12.32, 0.87 ± 0.28). The animals treated with imipramine (G3) showed a marginal increase in the maximum speed in zone (26.92 ± 4.14) and significant decrease ($p<0.05$) in the resting time in zone (1.25 ± 0.44) as compared to the UCS (G2). G4 showed altered maximum speed in zone (42.19 ± 8.63) and resting time in zone (1.73 ± 0.50) as compared to the G2.

The maximum speed in zone was increased by 64.34%, 71.95%, 20.84%, 79.28%, and 109.49% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2, while G7 was reported to be increased by 61.63% as compared with the G4 group. Similarly, the resting time in zone was significantly decreased by 86.27%, 82.74%, 77.89%, 53.86%, and 80.28% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2, while 74.17%, 67.51%, 58.39%, 13.17%, and 62.88% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G4. However, all the groups showed no significant changes in total distance travelled. Similarly, in case of latency in target zone record showed that 87.74%, 87.94%, 66.89%, 87.59%, and 81.88% decreased values in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2, while 48.21%, 49.06%, 47.59% and 23.47% decreased latency in target zone in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G4. However, entry in target zone was significantly increased by 215%, 110%, 190%, 125%, and 115% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2, while 43.18% and 31.82% increased values in the G5 and G6 group respectively as compared with the G4.

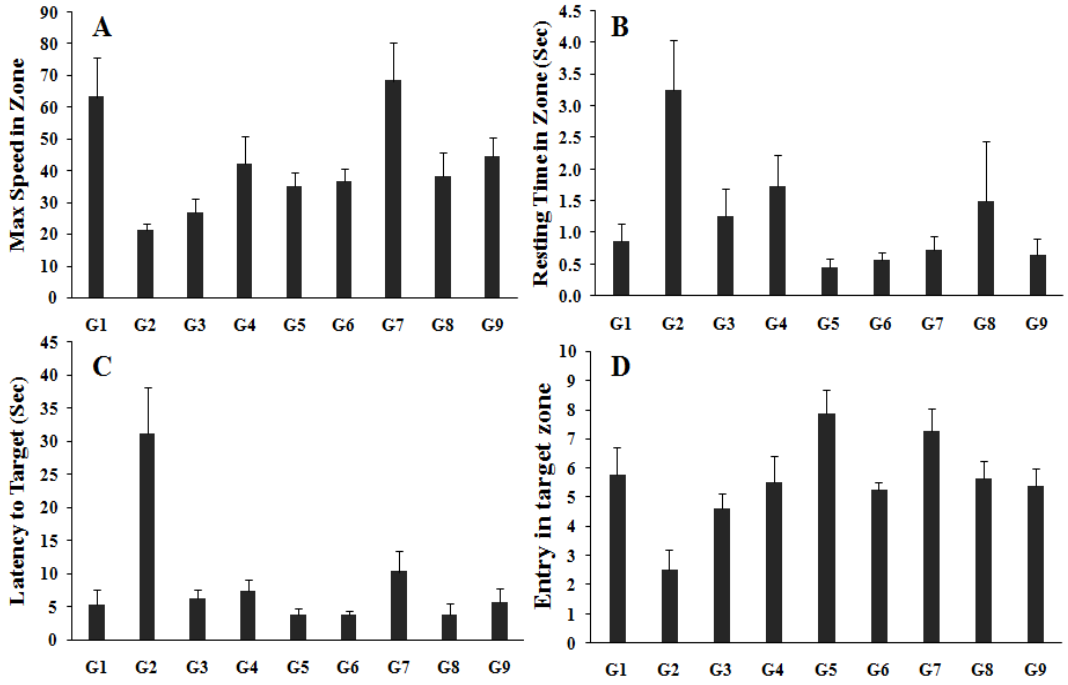

Figure 2: Effect of the test formulation using Morris water Maze test in various test groups from G1 to G9 in male Sprague Dawley rats. (A) Maximum speed in zone, (B) Resting time in zone, (C) Latency to Target (in sec), and (D) Entry in target zone. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Effect of test formulation for behaviour parameters using Forced Swim test

This test was ideal in case of testing the rodent behavioural such as depressant action. Besides it also aimed at rendering or preventing depressive-like states using inescapable transparent tank filled with water and their escape related mobility behaviour was measured in presence of test formulation using various experimental groups [44]. Forced Swim test results were compiled and represented graphically (Figure 3). The animals with UCS (G2) showed a significant increase (p<0.05) in the immobility time, (88.80 ± 10.96) and significant decrease in the number of climbing's, and swim time (31.60 ± 2.78, 211.20 ± 10.96, respectively) as compared to the normal control (G1) (41.10 ± 13.77, 51.50 ± 5.13, and 258.90 ± 13.77). The animals treated with imipramine (G3) showed a significant decrease (p<0.05) in the immobility time, (47.78 ± 10.25) and significant increase (p<0.05) in number of climbing's, and swim time, (60.63 ± 7.91 and 252.22 ± 10.25, respectively) as compared to the UCS (G2).

However, the number of climbing data suggested that G5, G6, G7, G8, and G9 groups showed an increased value by 101.34%, 146.04%, 93.04%, 141.30% and 125.39% respectively, as compared with the disease control G2 group, while 10.09% and 7.96% increased values were reported in case of G6 and G8 groups respectively as compared with the G4 group. However, the immobility time (sec.) data showed that G5, G6, G7, G8, and G9 groups showed a decreased time by 51.58%, 70.95%, 37.05%, 73.35%, and 58.11% respectively, as compared with the disease control G2 group, while 8.12%, 44.87%, 49.43%, and 20.51% decreased time values in the G5, G6, G8, and G9 groups respectively as compared with the G4 group. Similarly, the swim time data reported with increased values by 21.69%, 29.83%, 15.58%, 30.84%, and 24.43% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group, while 1.50%, 8.29%, 9.14%, and 3.79% decreased time values in the G5, G6, G8, and G9 groups respectively as compared with the G4 group.

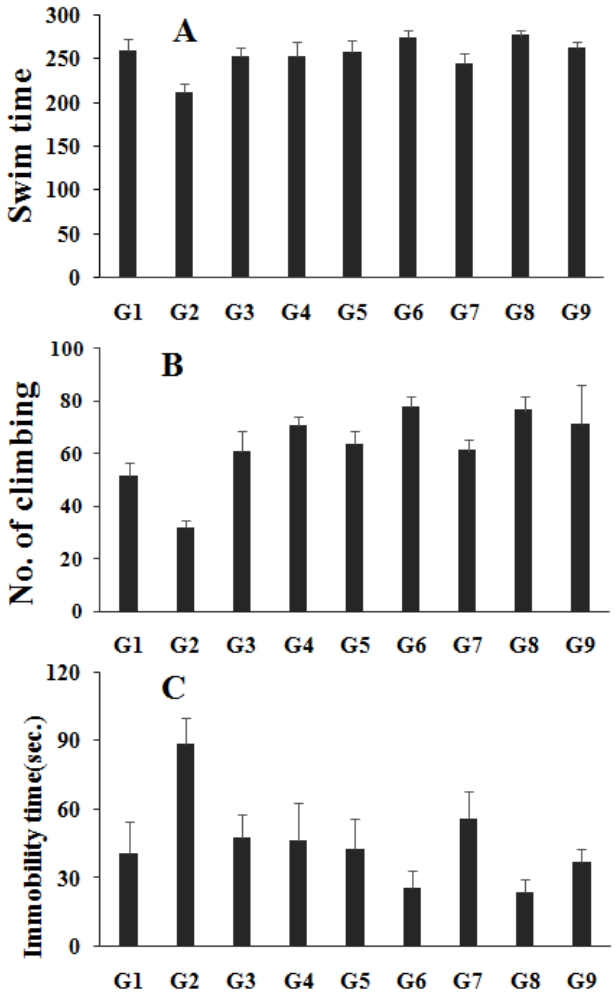

Figure 3: Effect of the test formulation in Forced Swim test in various test groups from G1 to G9 in male Sprague Dawley rats. (A) Swim time, (B) Number of climbing, and (C) Immobility time (in sec). G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Effect of test formulation for behaviour parameters using Open Field Test

OFT is used to identify locomotor and anxiety-like behaviour, anxiety-related emotional behaviours, and was very important for conventional and ethological parameters [45]. Open field test results were compiled and represented graphically (Figure 4).

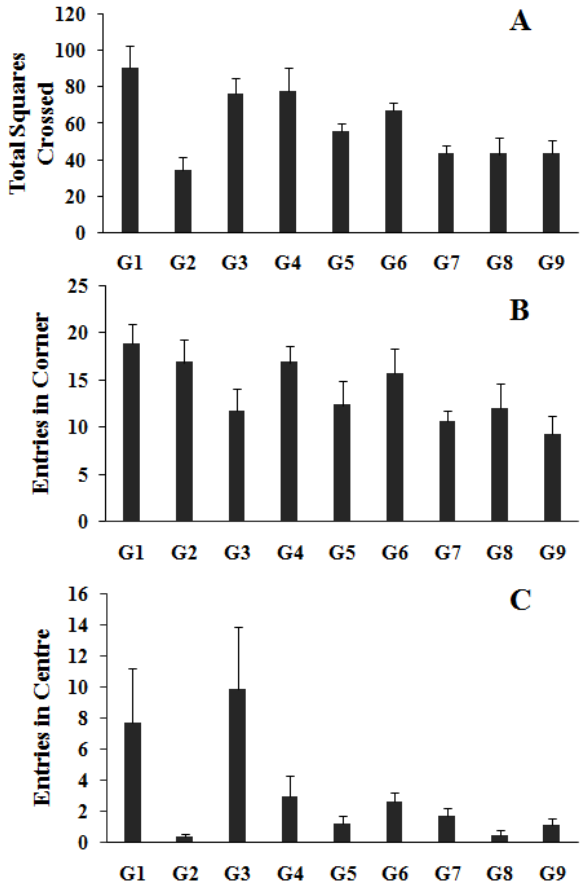

Figure 4: Effect of the test formulation Open field test in various test groups from G1 to G9 in male Sprague Dawley rats.

A. Total square crossed.
B. Entries in Corner.
C. Entries in centre.

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

The animals treated with UCS (G2) showed a significant decrease (p<0.001) in the total number of square crossed as well as entries in center zone, (34.38 ± 7.39 and 0.38 ± 0.18, respectively) indicating an anxiety behaviour as compared to the normal control (G1) (90.50 ± 12.42 and 7.75 ± 3.49). However, marginal increase in entries in corner zone of normal control (G1) group as compared with UCS (G2) group, (18.88 ± 2.11 and 16.88 ± 2.44). The animals treated with imipramine (G3) showed a significant increase (p<0.01) in the total number of square crossed as well as entries in center zone, (76.25 ± 8.43, 9.88 ± 4.04, respectively) as compared to the UCS (G2), while decreased entries in side corner square (11.75 ± 2.40) as compared to the UCS (G2).

The experimental group showed significant increased total number of square crossed by 62.18%, 94.55%, 26.55%, 25.80%, and 26.55% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group. Similarly, entries in the center zone was significantly increased by 233.33%, 600%, 366.67%, 34.11%, and 200% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group. However, entries in side corner square was significantly decreased by 26.67, 6.67%, 37.04%, 28.79%, and 45.19% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 and G4 group. However, number of defecation, number of pellets, and urination was also recorded, which was also improved after treatment with the Biofield Energy Treated test formulation.

Effect of test formulation for behaviour parameters using Elevated Plus Maze (EPM)

EPM is widely used for all type of behavioural assay, which depicts and validate the anti-anxiety effects that reflects the action of brain regions and mechanisms underlying the anxiety-related behaviour [46, 47]. Elevated Plus Maze results were compiled and represented graphically (Figure 5). Time spent in open and closed arm behaviour was reported in the UCS (G2) group showed a significant decrease (p<0.05) in the time spent into the open arm as well as increased time spent in closed arm, (1.00 ± 0.44 and 282.41 ± 5.90, respectively) indicating an anxiety behavior as compared to the normal control (G1) (26.00 ± 4.2 and 243.03 ± 13.20). The animals treated with imipramine (G3) showed a significant increased (p<0.05) values in the time spent into the open arm as well as decreased time spent in closed arm, (i.e. 26.00 ± 1.80 and 247.41 ± 5.30, respectively) as compared to the UCS (G2). Untreated test formulation to the untreated rats (G4) showed a increased value in the time spent into the open arm (28.00 ± 14.3) and marginal decrease in the time spent in closed arm (253.95 ± 20.8) as compared to the UCS (G2). However, data suggested that time spent into the open arm was significantly increased by 2458%, 1460%, 1750%, 1079%, and 969% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2, while time spent in closed arm was decreased by 9%, 8%, 3%, and 1% in the G5, G6, G7, and G9 groups respectively, as compared with the G2. Similarly, the entries in open arm behavior was reported and found to be increased by 375%, 275%, 400%, 243%, and 200% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2.

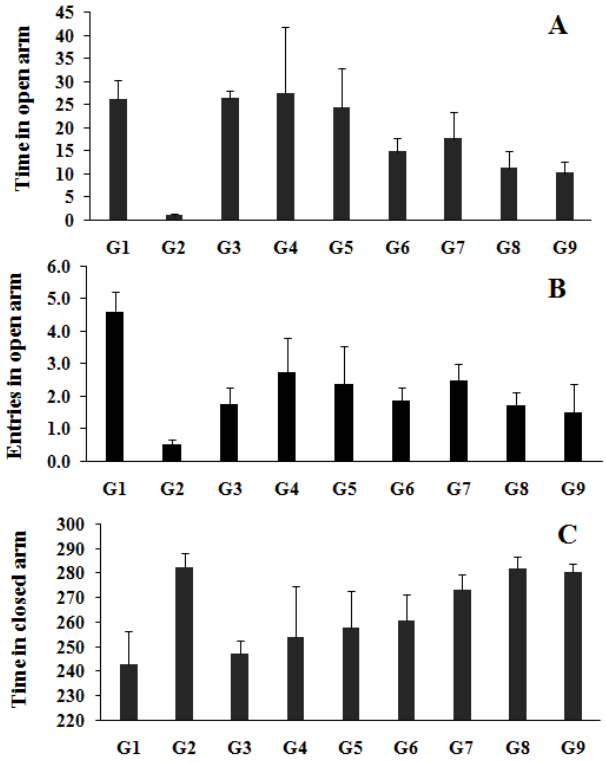

Figure 5: Effect of the test formulation for Elevated Plus Maze (EPM) in various test groups from G1 to G9 in male Sprague Dawley rats. (A) Time in open arm, (B) Entry in open arm, and (C) Time in closed arm. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30mg/kg); G4: (UCS + untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

The Effect of the test formulation for Sucrose preference test

This test is used for the measurement of stress-induced

anhedonia, assesses the level of depression, and was used for identification of depressive behaviour by the chronic stress [12,48]. Sucrose preference test results were compiled and represented graphically in Figure 6. The animals treated with the Unpredictable chronic stress (G2) showed significant decreases ($p<0.01$) in the percentage of sucrose preference (0.49 ± 0.02) as compared with the normal control (G1, 0.77 ± 0.04). Imipramine treatment (G3) significantly increased the sucrose preference ($p<0.01$) level (0.77 ± 0.04) as compared to the G2 indicating the reversed with treatment. Untreated test formulation to the untreated rats (G4) also showed a marginal increased percentage (0.55 ± 0.03) as compared to the G2. Similarly, the experimental test groups showed a significant increased level of sucrose preference by 37.09%, 35.63%, and 26.15% in the G5, G6, and G7 groups respectively as compared with the G2, while 20.79%, 19.51%, and 11.16% increased sucrose preference was reported in the G5, G6, and G7 groups respectively, as compared with the G4.

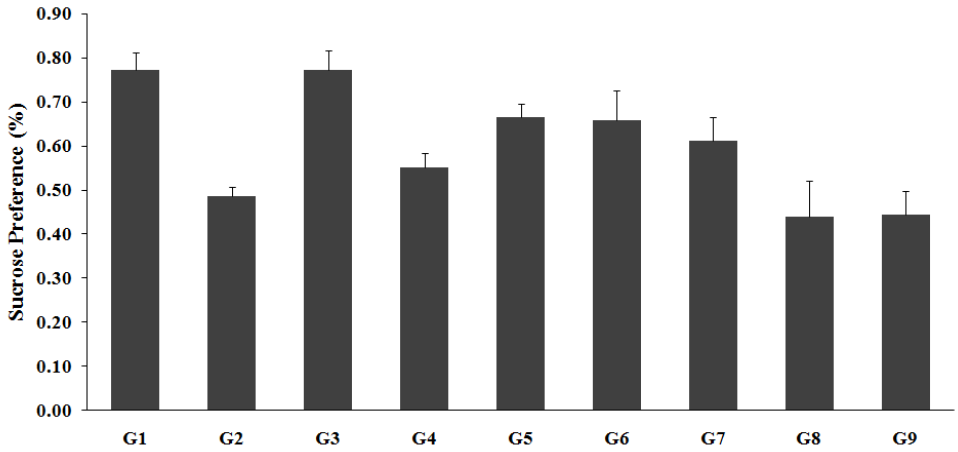

Figure 6: Effect of the test formulation for sucrose preference test in various test groups from G1 to G9 in male Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable Chronic Stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30mg/kg); G4: (UCS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

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

Conclusion

Behavioural parameters were tested in presence of Biofield Energy Treated test formulation among various experimental groups. The results were reported and concluded that Y maze test data showed time in start arm was significantly decreased by 76.92%, 34.33%, 24.63%, 55.52%, and 74.93% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. However, time in the novel arm was increased by 83.23%, 38.84%, 19.12%, 100.80%, and 72.40% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2, while time in the novel arm was increased by 110.02%, 59.13%, 36.54%, 130.15%, and 97.61% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G4. Another data of Y maze test showed that entry in start arm was significantly increased by 70.59% and 35.29% in the G6

and G7 groups, as compared with the G2, while entry in start arm was increased by 141.67% and 91.67% in the G6 and G7 group, respectively as compared with the G4. In addition, entry in explored arm was increased by 153.85% and 61.54% in the G6 and G7 groups, respectively as compared with the G4 group. Morris water Maze results showed that maximum speed in zone was increased by 64.34%, 71.95%, 20.84%, 79.28%, and 109.49% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2. Similarly, the resting time in zone was significantly decreased by 86.27%, 82.74%, 77.89%, 53.86%, and 80.28% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2, while 74.17%, 67.51%, 58.39%, 13.17%, and 62.88% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G4. Latency in target zone record showed that 87.74%, 87.94%, 66.89%, 87.59%, and 81.88% decreased values in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2, while entry in target zone was significantly increased by 215%, 110%, 190%, 125%, and 115% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2.

Forced swim test data showed that number of climbing data suggested that G5, G6, G7, G8, and G9 groups showed an increased value by 101.34%, 146.04%, 93.04%, 141.30% and 125.39% respectively, as compared with the disease control G2 group, while immobility time (sec.) data showed that G5, G6, G7, G8, and G9 groups showed a decreased time by 51.58%, 70.95%, 37.05%, 73.35%, and 58.11% respectively, as compared with the disease control G2 group. Similarly, swim time data showed increased values by 21.69%, 29.83%, 15.58%, 30.84%, and 24.43% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group. However, total number of square crossed was increased by 62.18%, 94.55%, 26.55%, 25.80%, and 26.55% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group. Similarly, entries in the center zone was also significantly increased by 233.33%, 600%, 366.67%, 34.11%, and 200% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the disease control G2 group. Elevated Plus Maze results showed that time spent into the open arm was significantly increased by 2458%, 1460%, 1750%, 1079%, and 969% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. In addition, the entries in open arm behaviour was reported and found to be increased by 375%, 275%, 400%, 243%, and 200% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2. On the other hand, sucrose preference test was significant increased by 37.09%, 35.63%, and 26.15% in the G5, G6, and G7 groups respectively, as compared with the G2. Thus, Biofield Energy Healing Treatment (The Trivedi Effect®) *per se* showed outstanding results with high efficacy in the preventive maintenance group, G6 as compared to the other preventive maintenance groups (G7, G8, and G9) in rat model study. It also helped to slow down the disease progression and disease related complications of the overall animal's health. These data suggested that Biofield Energy Treatment *per se* and/or Biofield Energy Treated Test formulation in combination would be the best treatment strategies in order to prevent and protect from the occurrence of any type of diseases.

Therefore, the Biofield Energy Treatment might act as a preventive maintenance therapy in order to maintain good health, or full restoration of health or improve the overall health and quality of life in human. This therapy might also reduce the severity of any type of stress related disorders and its progression rate and can be used in both before and after the manifestation of most of the immunity related disorders in healthy, unhealthy, and ill peoples such as human body immune responses, enhance resistance towards diseases, Cancer, Hashimoto's thyroiditis, allergies, lethargic conditions, energy booster action, improve exercise capacity in heart related disorders, Crohn's disease, autoimmune diseases, and its various immune deficiency diseases. Overall, the data suggested the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* in showed significant action on behavioural parameters. This test formulation also can be used against Addison Disease, Multiple Sclerosis, Myasthenia Gravis, Rheumatoid Arthritis, Crohn's Disease, Vitiligo, and Alopecia Areata, as well as various inflammatory disorders such as Ulcerative Colitis, Dermatitis, Hepatitis, Diverticulitis, Mental Disorders, Parkinson's and Other Movement Disorders, Stroke and in the improvement of overall health and quality of life.

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References

1. Lezak KR, Missig G, Carlezon WA Jr (2017) Behavioral methods to study anxiety in rodents. Dialogues Clin Neurosci 19(2): 181-191.

2. Beck KD, Luine VN (2002) Sex differences in behavioral and neurochemical profiles after chronic stress: Role of housing conditions. Physiol Behav 75(5): 661-673.

3. Handley SL, Mithani S (1984) Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn Schmiedebergs Arch Pharmacol 327(1): 1-5.

4. Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem

Behav 24(3): 525-529.

5. File SE (1985) Animal models for predicting clinical efficacy of anxiolytic drugs: social behaviour. Neuropsychobiology 13(1-2): 55-62.

6. Sestakova N, Puzserova A, Kluknavsky M, Bernatova I (2013) Determination of motor activity and anxiety-related behaviour in rodents: methodological aspects and role of nitric oxide. Interdiscip Toxicol 6(): 126-135.

7. Conrad CD, Grote KA, Hobbs RJ, Ferayorni A (2003) Sex differences in spatial and non-spatial Y-maze performance after chronic stress. Neurobiol Learn Mem 79(1): 32-40.

8. Conrad CD, Galea LAM, Kuroda Y, McEwen BS (1996) Chronic stress impairs rat spatial memory on the Y-Maze, and this effect is blocked by tianeptine pretreatment. Behavioral Neuroscience 110(6): 1321-1334.

9. Kraeuter AK, Guest PC, Sarnyai Z (2019) The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. Methods Mol Biol 1916: 105-111.

10. Yankelevitch-Yahav R, Franko M, Huly A, Doron R (2015) The forced swim test as a model of depressive-like behavior. J Vis Exp (97): 10.3791/52587.

11. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, et al. (2012) The mouse forced swim test. J Vis Exp 59: e3638.

12. Teegarden S (2012) Behavioral Phenotyping in Rats and Mice. Mater Methods 2: 122.

13. Belovicova K, Bogi E, Csatlosova K, Dubovicky M (2017) Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol 10(1): 40-43.

14. D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain Res Rev 36(1): 60-90.

15. Nunez J (2008) Morris Water Maze Experiment. J Vis Exp 19: 897.

16. Byrne JH, Voogt M, Turner KM, Eyles DW, McGrath JJ, et al. (2013) The impact of adult vitamin D deficiency on behaviour and brain function in male Sprague-Dawley rats. PLoS One 8(8):e71593.

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

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

19. 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.

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

21. Kang S, Min H (2012) Ginseng, the 'Immunity Boost': The Effects of Panax ginseng on Immune System. J Ginseng Res 36(4): 354-368.

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

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

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

25. 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.

26. Fan K wai (2005) National Center for Complementary and Alternative Medicine Website. J Med Libr Assoc 93: 410-412.

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

28. Branton A, Jana S (2017) The influence of energy of consciousness healing treatment on low bioavailable resveratrol in male *Sprague Dawley* rats. International Journal of Clinical and Developmental Anatomy 3(3): 9-15.

29. Branton A, Jana S (2017) The use of novel and unique biofield energy healing treatment for the improvement of poorly bioavailable compound, berberine in male *Sprague Dawley* rats. American Journal of Clinical and Experimental Medicine 5(4): 138-144.

30. Trivedi MK, Tallapragada RM (2008) A transcendental to changing metal powder characteristics. Met Powder Rep 63(9): 22-28, 31.

31. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O (2015) Studies of the atomic and crystalline characteristics of ceramic oxide nano powders after biofield treatment. IndEng Manage 4: 161.

32. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated

alphonso mango (*Mangifera indica* L.). Journal of Food and Nutrition Sciences 3(6): 245-250.

33. Nayak G, Altekar N (2015) Effect of biofield treatment on plant growth and adaptation. J Environ Health Sci 1: 1-9.

34. Trivedi MK, Branton A, Trivedi D, Nayak G, Charan S, Jana S (2015) Phenotyping and 16S rDNA analysis after biofield treatment on *Citrobacter braakii*: A urinary pathogen. J Clin Med Genom 3(1): 129.

35. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) Evaluation of biofield modality on viral load of Hepatitis B and C viruses. J Antivir Antiretrovir 7(3): 083-088.

36. Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, et al. (2017) A Systematic study of the biofield energy healing treatment on physicochemical, thermal, structural, and behavioral properties of magnesium gluconate. International Journal of Bioorganic Chemistry 2(3): 135-145.

37. Kinney JP, Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2017) Overall skin health potential of the biofield energy healing based herbomineral formulation using various skin parameters. American Journal of Life Sciences 5(2): 65-74.

38. Singh J, Trivedi MK, Branton A, Trivedi D, Nayak G, et al. (2017) Consciousness energy healing treatment based herbomineral formulation: A safe and effective approach for skin health. American Journal of Pharmacology and Phytotherapy 2: 1-10.

39. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J Integr Oncol 4: 141.

40. Anagnos D, Trivedi K, Branton A, Trivedi D, Nayak G, et al. (2018) Influence of biofield treated vitamin D_3 on proliferation, differentiation, and maturation of bone-related parameters in MG-63 cell-line. International Journal of Biomedical Engineering and Clinical Science 4: 6-14.

41. Lee AC, Trivedi K, Branton A, Trivedi D, Nayak G, et al. (2018) The potential benefits of biofield energy treated vitamin D3 on bone mineralization in human bone osteosarcoma cells (MG-63). International Journal of Nutrition and Food Sciences 7: 30-38.

42. Kraeuter AK, Guest PC, Sarnyai Z (2019) The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. Methods Mol Biol 1916: 105-111.

43. Barnhart CD, Yang D, Lein PJ (2015) Using the Morris water maze to assess spatial learning and memory in weanling mice. PLoS One 10(4): e0124521.

44. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, et al. (2012) The mouse forced swim test. J Vis Exp (59): e3638.

45. Seibenhener ML, Wooten MC (2015) Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp 6(96): e52434.

46. Walf AA, Frye CA (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2(2): 322–328.

47. Biedermann SV, Biedermann DG, Wenzlaff F, Kurjak T, Nouri S, et al. (2017) An elevated plus-maze in mixed reality for studying human anxiety-related behavior. BMC Biol 15(1):125.

48. MUCSat R and Willner P (1989) Effects of dopamine receptor antagonists on sucrose consumption and preference. Psychopharmacology (Berl) 99(1): 98-102.