impairment [25-27]. UCS model significantly increased the level of corticosterone, and the test formulation significantly maintains the level of hormone. The effect of test formulation on the level of plasma corticosterone was determined and the data are presented in **Figure 1**. Corticosterone level in unpredictable chronic stress (UCS) G2 group was found to be 176.09 ± 13.1 ng/mL, which was significantly ($p \leq 0.001$) increased by 294.4% as compared with the control (G1, 44.46 ± 13.2 ng/mL). Imipramine treatment (G3) significantly ($p \leq 0.01$) decreased the corticosterone level (94.88 ± 22.1 ng/mL) by 46.1% as compared to the G2. Untreated test formulation to the untreated rats (G4) showed corticosterone level as 237.64 ± 64.7 ng/mL. Biofield Energy Treated Test formulation to the untreated rats (G5) showed decreased level (119.35 ± 22.5 ng/mL) by 32.2% and 49.8% as compared to the G2 and G4 groups, respectively. Biofield Energy Treatment *per se* to the rats (G6) significantly decreased the corticosterone level (58.85 ± 7.0 ng/mL) by 66.6% ($p \leq 0.01$) and 75.2% ($p \leq 0.05$) as compared to the G2 and G4 groups, respectively. 15 days pre-treatment of Biofield Energy Treated Test formulation (G7) showed significant decreased level (138.23 ± 22.6 ng/mL) by 21.5% and 41.8% as compared to the G2 and G4 groups, respectively. 15 days pre-treatment of Biofield Energy Treated Test formulation to the Biofield treated rats (G8) group showed significant decreased corticosterone level (123.79 ± 21.2 ng/mL) by 29.7% and 47.9% as compared to the G2 and G4 groups, respectively. Untreated Test formulation to the Biofield Energy Treated *per se* rats (G9) decreased (169.42 ± 23.9 ng/mL) by 3.8% and 28.7% as compared to the G2 and G4 groups, respectively.

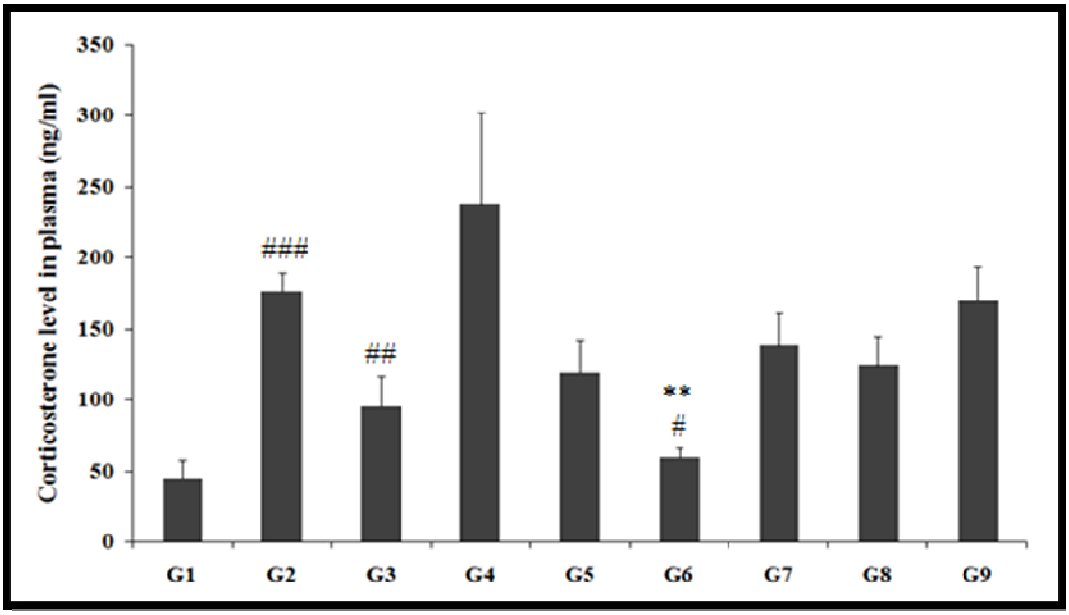

Figure 1. Effect of the test formulation on the level of plasma corticosterone in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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). $^{\#}p \leq 0.05$ *vs.* G4, $^{\#\#}p \leq 0.01$ *vs.* G2, $^{\#\#\#}p \leq 0.001$ *vs.* G1, $^{**}p \leq 0.01$ *vs.* G2.

2. Effect of the Test Formulation on Plasma Angiotensin-II

One of the important stress hormones is the angiotensin II, which is significantly increased in acute and chronic stress. This can lead to many clinical implications with respect to the kidney and heart physiology-related to inflammation, tissue injury, autoimmunity, oxidative stress and aging [28,29]. The effect of test formulation on the level of plasma angiotensin II was determined and the results are compiled in the **Figure 2**. Plasma angiotensin II level in UCS group (G2) was 55.67 ± 5.3 pg/mL, which was significantly ($p \leq 0.01$) increased by 67.9% in comparison with the normal control (G1) 33.15 ± 3.0 pg/mL. Imipramine treatment (G3) significantly ($p \leq 0.001$) decreased the plasma angiotensin II level (31.80 ± 3.9 pg/mL) by 42.9% as compared to the G2. G4 group was reported with significantly ($p \leq 0.001$) decreased plasma angiotensin II level (37.57 ± 1.5 pg/mL) by 32.5% as compared to the G2. Similarly, G5 (33.05 ± 1.5 pg/mL) group showed significant ($p \leq 0.001$) decreased of percentage of plasma angiotensin II by 40.6% and 12.0% as compared to the G2 and G4 groups, respectively. G6 group showed significantly ($p \leq 0.001$) decreased plasma angiotensin

II (30.07 ± 2.7 pg/mL) by 46% and 20% as compared to the G2 and G4 groups, respectively. G7 group showed significantly decreased plasma angiotensin II (35.70 ± 3.6 pg/mL) by 35.9% ($p \leq 0.001$) and 5% as compared to the G2 and G4 groups, respectively. G8 (43.68 ± 4.1 pg/mL) and G9 (35.91 ± 3.8 pg/mL) group showed significantly decreased plasma angiotensin II by 21.5% and 35.5%, respectively as compared to the G2. G9 group showed decreased plasma angiotensin II by 4.4% as compared to the G4.

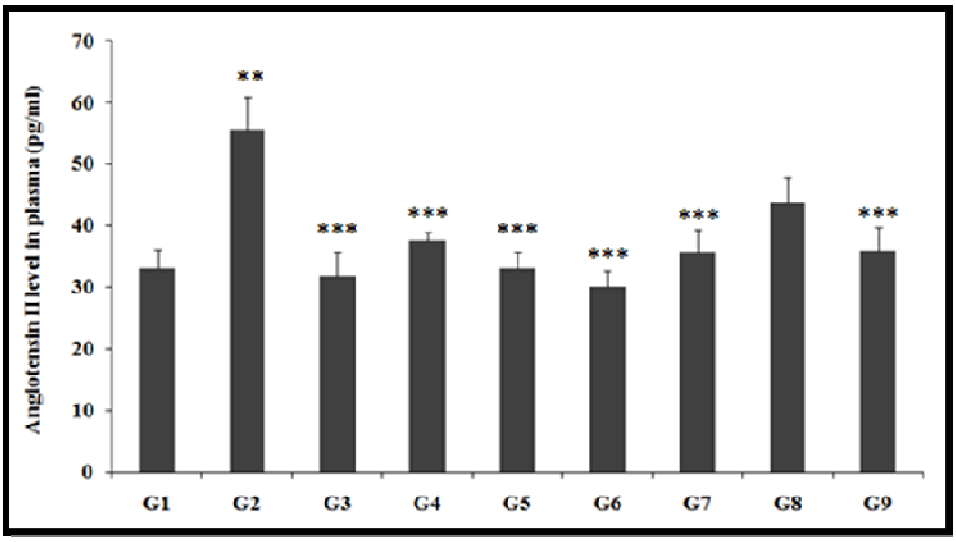

Figure 2. Effect of the test formulation on the level of plasma angiotensin-II in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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). $^{**}p \leq 0.01$ *vs.* G1 and $^{***}p \leq 0.001$ *vs.* G2.

3. Effect of the Test Formulation on Plasma Noradrenaline

The role of noradrenaline in stress or psychological conditions was well established and they play a vital role in cardiovascular diseases [30,31]. Many disorders are directly linked with clinical pathologies such as chronic active hepatitis, asthmatics, Crohn's disease, ulcerative colitis, trigeminal neuralgia, chronic relapsing hepatitis, multiple sclerosis, systemic lupus erythematous (SLE), and rheumatoid arthritis (RA). The effect of test formulation on the level of plasma noradrenaline was determined and the results are compiled in the **Figure 3**. Plasma noradrenaline level in the UCS group (G2) was 239.57 ± 22.3 pg/mL, which was increased by 29.1% as compared with the normal control (G1, 185.64 ± 36.6 pg/mL). Imipramine treatment (G3) decreased the plasma noradrenaline level (134.42 ± 26.0 pg/mL) by 43.9% as compared to the G2. Untreated test formulation to untreated rats (G4) showed value as 305.69 ± 41.8 pg/mL. Besides, G6, G7, and G8 groups showed decreased the plasma noradrenaline level by 13.6%, 20.2%, and 28.4%, respectively as compared to the G4 group.

4. Effect of the Test Formulation on Plasma Epinephrine

The effects of mental and physical stress have significant impact on plasma epinephrine. It has been reported that increases in circulating epinephrine or adrenaline have been linked with various level of physical sensations (symptoms) which are associated with acute and chronic stress [32]. The effect of the test formulation on the level of plasma epinephrine was determined and the results are compiled in the **Figure 4**. Plasma epinephrine level in the UCS group (G2) was 86.94 ± 13.17 pg/mL, which was significantly ($p \leq 0.01$) increased by 132.2% in comparison with the normal control (G1, 37.44 ± 5.37 pg/mL) group. Imipramine treatment (G3) significantly ($p \leq 0.01$) decreased the plasma epinephrine level (33.6 5 ± 5.50 pg/mL) by 61.3% as compared to the G2. G4 group was reported with decreased plasma epinephrine level (58.14 ± 10.57pg/mL) by 33.1% as compared to the G2. Similarly, G5 (41.32 ± 7.81 pg/mL) group showed significant ($p \leq 0.05$) decreased percentage of plasma epinephrine by 51.9% and 28.1% as compared to the G2 and G4 groups, respectively. G6 group showed significantly ($p \leq 0.05$) decreased plasma epinephrine (41.82 ± 8.12 pg/mL) by 51.9% and 28.1% as compared to the G2 and G4 groups, respectively. G7 group showed decreased plasma epinephrine (54.20 ± 19.23 pg/mL) by 37.7% and 6.8% as compared to the G2 and G4 groups, respectively. G8 (64.71 ± 16.33 pg/mL) and G9 (56.21 ± 11.96 pg/mL) groups

showed decreased plasma epinephrine by 25.6% and 35.4%, respectively as compared to the G2 group.

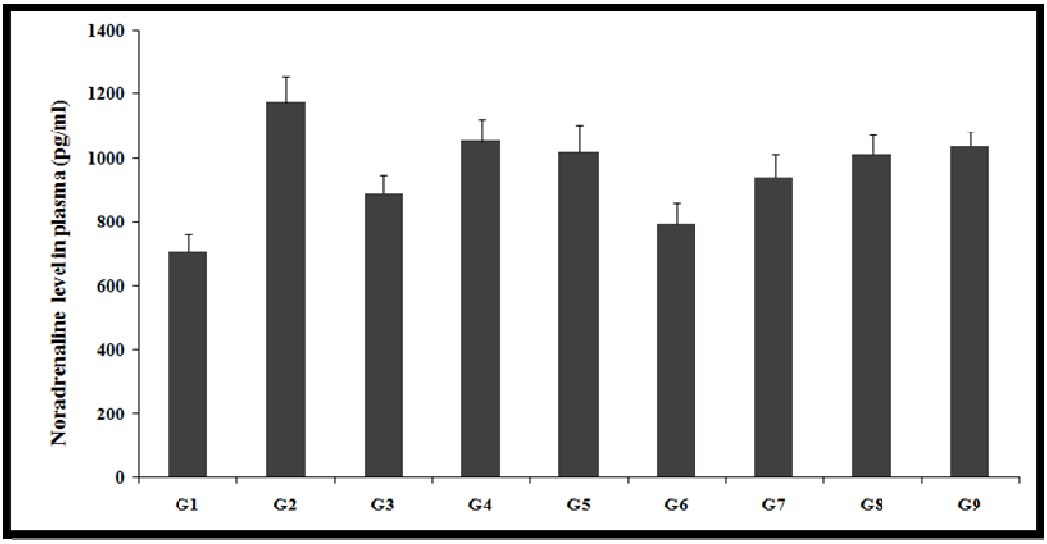

Figure 3. Effect of the test formulation on the level of plasma noradrenaline in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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).

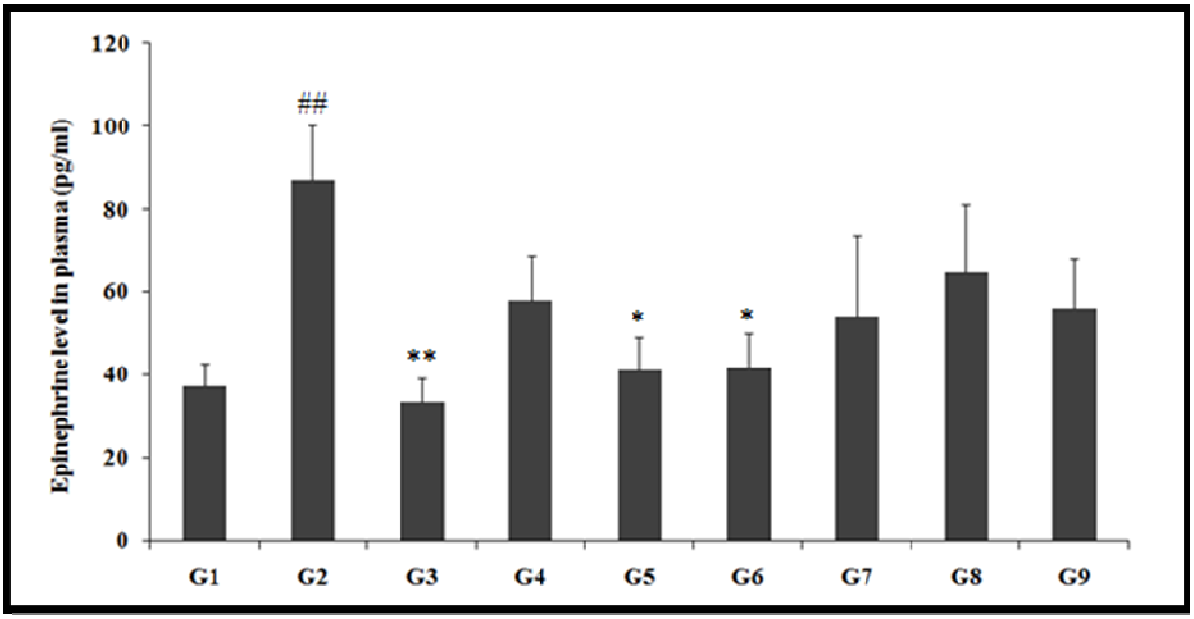

Figure 4. Effect of the test formulation on the level of plasma epinephrine in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (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). *$p \leq 0.05$ *vs.* G2, **$p \leq 0.01$ *vs.* G2, $^{\#\#}p \leq 0.01$ *vs.* G1.

5. Effect of the Test Formulation on CSF Norepinephrine

Stress disorder along with blood pressure and cerebrospinal fluid also contribute to affect the level of norepinephrine in CSF [33]. The effect of the test formulation on the level of CSF norepinephrine was determined and the results are compiled in the **Figure 5**. CSF norepinephrine level in the UCS group (G2) was 1172.27 ± 82.73 pg/mL, which was significantly ($p \leq 0.001$) increased by 65.5% in comparison with the normal control (G1, 708.19 ± 52.98 pg/mL). Imipramine treatment (G3) significantly ($p \leq 0.05$) decreased the CSF nor-epinephrine level (888.08 ± 58.76 pg/mL) by 24.2% as compared to the G2. G4 group was reported with decreased CSF norepinephrine level (1052.01 ± 71.23pg/mL) by 10.3% as compared to the G2. Similarly, G5 (1017.57 ± 84.78 pg/mL) group showed decreased percentage of CSF norepinephrine by 13.27% and 3.3% as compared to the G2 and G4 groups, respectively. G6 group showed significantly ($p \leq 0.05$) decreased CSF norepinephrine (795.34 ± 65.66 pg/mL) by 32.2% and 24.4% as compared to the G2 and G4 groups, respectively. G7 group showed decreased CSF norepinephrine (941.60 ± 69.78 pg/mL) by 19.7% and 10.5% as compared to the G2 and G4 groups, respectively. G8 (1011.93 ± 61.13 pg/mL) group showed decreased CSF nor-epinephrine by 13.7% and 3.8% as compared to the G2 and G4, respectively. G9 (1037.23 ± 43.81 pg/mL) group showed decreased CSF nor-epinephrine by 11.5% as compared to the G2.

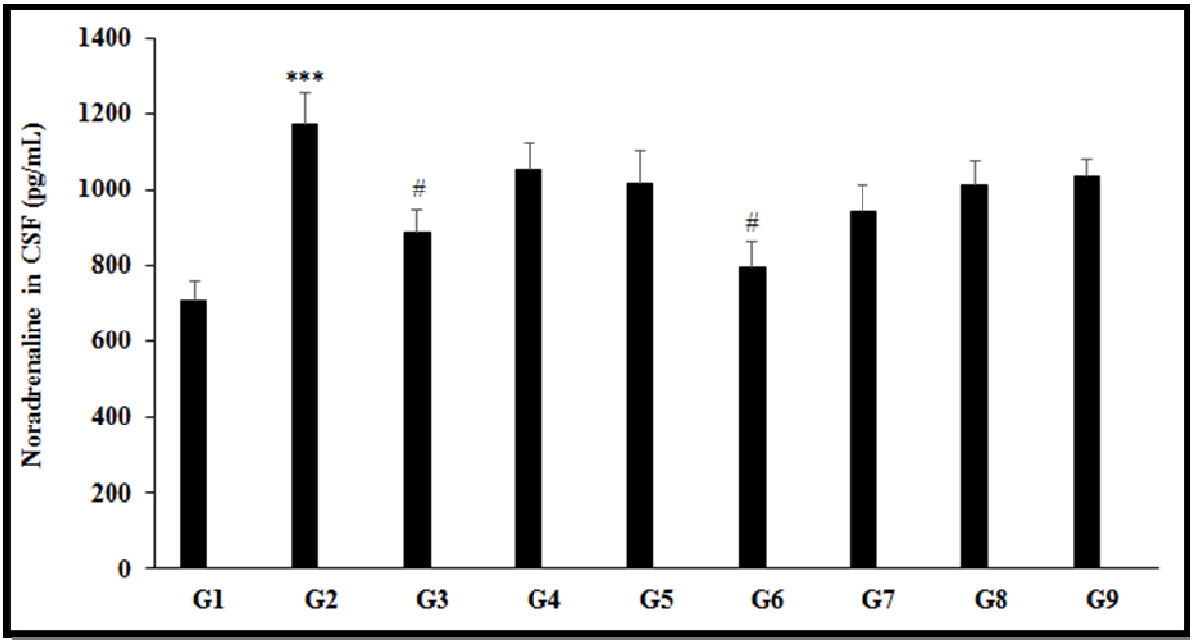

Figure 5. Effect of the test formulation on the level of nor-epinephrine in cerebro-spinal fluids (CSF) of Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated 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). $^{\#}p \leq 0.05$ *vs.* G2 and $^{***}p \leq 0.01$ *vs.* G1.

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 a significant slowdown of disease progression and all other disease-related 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 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 beneficial to prevent and protect from the occurrence of any type of disease in the rat model. The data indicated that this therapy could act as a preventive maintenance therapy to prevent the occurrence of disease, slowdown the disease progression when disease-related complications are present which will ultimately improve the overall health and quality of life.

CONCLUSIONS

The present study demonstrated the effect of Biofield Energy Treated test formulation and Biofield Energy *per se* for the estimation of stress hormone that showed significant improved maintenance of the hormonal level, which have significant clinical role in stress-related disorders. Plasma corticosterone level was significantly decreased by 49.8%, 75.2%, 41.8%, 47.9% and 28.7% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the untreated test formulation (G4) group. The data of plasma angiotensin-II showed significant reduced plasma level by 40.6% ($p \leq 0.001$), 46% ($p \leq 0.001$), 35.9% ($p \leq 0.001$), 21.5%, and 35.5% ($p \leq 0.001$) in the G5, G6, G7, and G9 groups, respectively as compared with the G4. In addition, plasma noradrenaline level was reduced by 13.6%, 20.2%, and 28.4% in the G6, G7, and G8 groups, respectively compared to the G4. Plasma epinephrine level was significantly reduced by 51.9% ($p \leq 0.05$), 51.9% ($p \leq 0.05$), 37.7%, 25.6%, and 35.4% in the G5, G6, G7, G8, and G9 groups, respectively compared to the G2 group. Norepinephrine level in CSF was significantly decreased by 13.27%, 32.2% ($p \leq 0.05$), 19.7%, 13.7%, and 11.5% in the G5, G6, G7, G8, and G9 groups, respectively compared to the G2 group. Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed the best results with respect to different beneficial efficacy and biomarker parameters in the preventive maintenance group, G6, as compared to the other preventive maintenance groups (G7, G8, and G9) in the rat model study. The Biofield Energy Healing Treatment also helped to slowdown the disease progression and disease-related complications impacting the overall animals' health. These data suggested that Biofield Energy Treatment *per se* and Biofield Energy Treated Test formulation in combination would be the best treatment strategy to prevent and protect from the occurrence of any type of diseases. Therefore, the Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* might be effective in healthy humans, when used as a preventive maintenance therapy to sustain good health, to boost overall health, promote healthy aging and increase quality of life. In the presence of disease, the Biofield Energy therapy might reduce the severity of any acute/chronic disease (such as auto-immune-related and inflammatory disorders) and / or slow the disease progression. This test formulation can be used against systemic lupus erythematosus, fibromyalgia, Addison disease, multiple sclerosis, myasthenia gravis, pernicious anemia, aplastic anemia, psoriasis, rheumatoid arthritis, Crohn's disease, vitiligo, chronic fatigue syndrome and alopecia Areata, as well as inflammatory disorders such as ulcerative colitis, atherosclerosis, dermatitis, hepatitis, and diverticulitis. However, Biofield Energy Healing Treated test formulation and Biofield Energy Healing Treatment *per se* can also be used in the prevention of brain disorders such as Alzheimer's disease, dementias, brain cancer, epilepsy and other seizure disorders, mental disorders, Parkinson's and other movement disorders, stroke and transient ischemic attack and in the improvement of overall health and quality of life.

REFERENCES

1. Grant BF, Hasin DS, Stinson FS, Dawson DA, Ruan JW, et al. (2005) Prevalence, correlates, co-morbidity, and comparative disability of DSM-IV generalized anxiety disorder in the USA: Results from the National epidemiologic survey on alcohol and related conditions. Psychol Med 35: 1747-1759.
2. Kendler KS, Karkowski LM, Prescott CA (1998) Stressful life events and major depression: Risk period, long-term contextual threat, and diagnostic specificity. J Nerv Ment Dis 186: 661-669.
3. Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE (2007) The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain Cogn 65(3): 209-237.
4. McEwen BS (2006) Protective and damaging effects of stress mediators: Central role of the brain. Dialogues Clin Neurosci 8(4): 367-381.
5. Kalivas PW, Duffy P (1995) Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. Brain Res 675(1): 325-328.
6. Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, et al. (2009) Chronic stress causes front striatal reorganization and affects decision-making. Science 325(5940): 621-625.
7. Erb S, Stewart J (1999) A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. J Neurosci 19: RC35.
8. Chappell P, Smith M, Kilts C, Bissette G, Ritchie J, etal. (1986) Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat brain regions after acute and chronic stress. J Neurosci 6(10): 2908-2914.
9. Yang G, Wan Y, Zhu Y (1996) Angiotensin II--an important stress hormone. Biol Signal 5(1): 1-8.
10. Yang G, Xi ZX, Wan Y, Wang H, Bi G (1993) Changes in circulating and tissue angiotensin II during acute and chronic stress. Biol Signal 2(3): 166-172.
11. Sadowski RN, Jackson GR, Wieczorek L, Gold PE (2009) Effects of stress, corticosterone, and epinephrine administration on learning in place and response tasks. Behav Brain Res 205(1): 19-25.
12. Seki K, Yoshida S, Jaiswal MK (2018) Molecular mechanism of noradrenaline during the stress-induced major depressive disorder. Neural Regen Res 13(7): 1159-1169.

13. Rubik B (2002) The biofield hypothesis: Its biophysical basis and role in medicine. J Altern Complement Med 8: 703-717.

14. Jain S, Hammerschlag R, Mills P, Cohen L, Krieger R, et al. (2015) Clinical studies of biofield therapies: Summary, methodological challenges, and recommendations. Glob Adv Health Med 4: 58-66.

15. Evans M, Shaw A, Thompson EA (2007) Decisions to use complementary and alternative medicine (CAM) by male cancer patients: Information-seeking roles and types of evidence used. BMC Complement Altern Med 7: 25.

16. Gu S, Pei J (2017) Innovating Chinese herbal medicine: From traditional health practice to scientific drug discovery. Front Pharmacol 8: 381.

17. O'Mathúna D (2001) The best of both approaches. The role of science in complementary and alternative medicine. EMBO Rep 2(12): 1054-1057.

18. Kumar MT, Alice B, Dahryn T, Snehasis J (2021) Effect of consciousness energy healing treatment on the metal profile and properties of tellurium. Eng Technol Open Acc 3(5): 555623.

19. 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.

20. 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.). J Food Nutr Sci 3: 245-250.

21. 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 Neurol Brain Disord 5(2): 2021.

22. Kumar MT, Snehasis J (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.

23. 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.

24. 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.

25. Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, et al. (2003) Chronic stress and obesity: A new view of 'comfort food'. Proc Natl Acad Sci USA 100: 11696-11701.

26. Martin LB (2009) Stress and immunity in wild vertebrates: Timing is everything. Gen Comp Endocrinol 163: 70-76.

27. de Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: From adaptation to disease. Nat Rev Neurosci 6: 463-475.

28. Benigni A, Cassis P, Remuzzi G (2010) Angiotensin II revisited: New roles in inflammation, immunology and aging. EMBO Mol Med 2(7): 247-257.

29. Arregui A, Perry EK, Rossor M, Tomlinson BE (1982) Angiotensin converting enzyme in Alzheimer's disease increased activity in caudate nucleus and cortical areas. J Neuro Chem 38: 1490-1492.

30. Hollenberg NK, Williams GH, Adams DF (1981) Essential HT: Abnormal renal vascular and endocrine responses to a mild psychological stimulus. Hypertension 3: 11-17.

31. Folkow B, Di Bona GF, Hjelmdahl P, Toren PH, Wallin BG (1983) Measurements of plasma norepinephrine concentrations in human primary hypertension. Hypertension 5: 399-403.

32. Paran E, Neumann L, Cristal N (1992) Effects of mental and physical stress on plasma catecholamine levels before and after β-adrenoceptor blocker treatment Eur J Clin Pharmacol 43: 11.

33. Strawn JR, Ekhator NN, Horn PS, Baker DG, Geracioti TD Jr (2004) Blood pressure and cerebrospinal fluid norepinephrine in combat-related posttraumatic stress disorder. Psychosom Med 66(5): 757-759.