compared to the G2. The untreated test formulation to the untreated rats (G4) showed decreased liver ATP (10.68 ± 0.3 nmol/well) by 9.1% as compared to G2. G5 group showed decreased liver ATP level (10.41 ± 0.4 nmol/well) by 11.4% as compared to *G*2. G6 group showed decreased liver ATP level (10.34 ± 0.4 nmol/well) by 12% and 3.2% as compared to the G2 and G4 groups, respectively. G7 group showed decreased ATP level (11.03 ± 0.2 nmol/well) by 6.1% as compared to the G2; while increased by 3.3% as compared to the G4 group. G8 group animals showed significantly decreased ATP level (9.89 ± 0.3 nmol/well) by 15.8% and 7.4% as compared to the G2 and G4 groups, respectively. G9 group showed decreased ATP level (11.06 ± 0.3 nmol/well) by 5.8% as compared to the G2; while increased by 3.5% as compared to the G4 group.

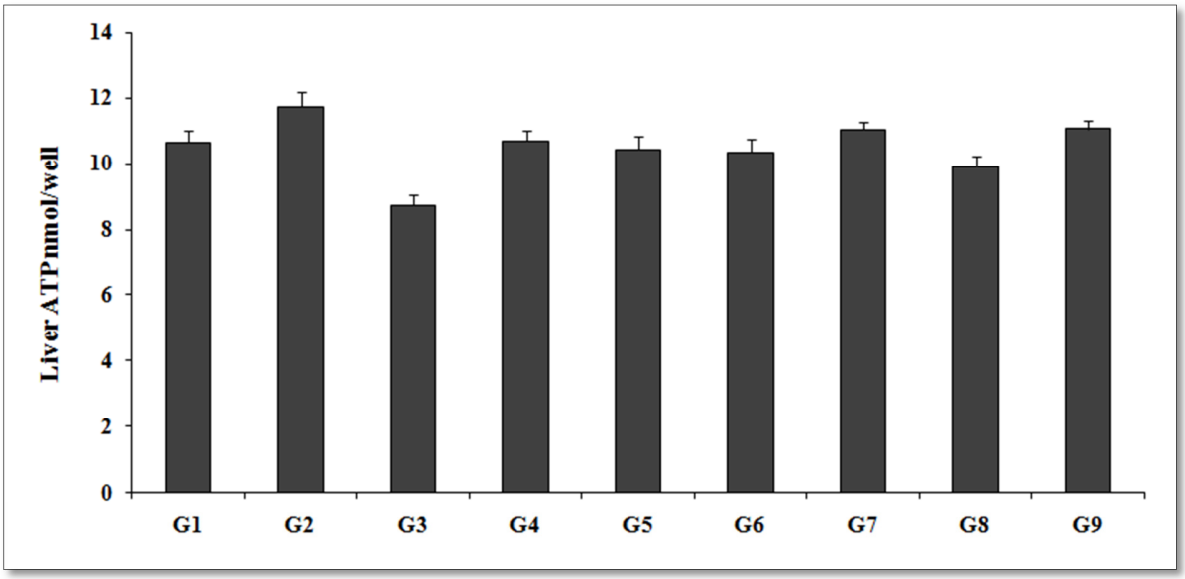

Figure 2. The effect of the test formulation on the level of liver ATP in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + 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).

Estimation of ATP in Muscle

Muscle fatigue is one the common non-specific symptom-related with muscle ATP. ATPase has reported to be useful in management of tropomyosin for relaxation. Fatigue can be managed with the help of intracellular ATP. Muscular performance would be optimum in presence of muscle ATP and its energy [41,42]. The level of ATP in muscle was measured in all the experimental groups are graphically presented in the **Figure 3**. The data suggested that unpredictable chronic stress group (G2) showed muscle ATP level was 1.93 ± 0.3 nmol/well, *i.e.,* increased by 128.9% as compared with the control (G1, 0.84 ± 0.1 nmol/well). Imipramine treatment (G3) showed decreased muscle ATP level (0.74 ± 0.1 nmol/well) by 61.9% as compared to the G2. The untreated test formulation to the untreated rats (G4) showed decreased muscle ATP (0.78 ± 0.2 nmol/well) by 59.4% as compared to the G2. G5 group showed decreased muscle ATP level (0.72 ± 0.1 nmol/well) by 62.5% and 7.6% as compared to the G2 and G4 groups, respectively. G6 group showed decreased muscle ATP level (0.50 ± 0.0 nmol/well) by 74.2% and 36.4% as compared to the G2 and G4 groups, respectively. G7 group showed decreased ATP level (0.50 ± 0.0 nmol/well) by 74.3% and 36.8% as compared to the G2 and G4 groups, respectively. G8 group animals showed decreased ATP level (0.62 ± 0.0 nmol/well) by 68% and 21.1% as compared to the G2 and G4 groups, respectively. G9 group showed decreased ATP level (0.59 ± 0.0 nmol/well) by 69.4% and 24.6% as compared to the G2 and G4 groups, respectively.

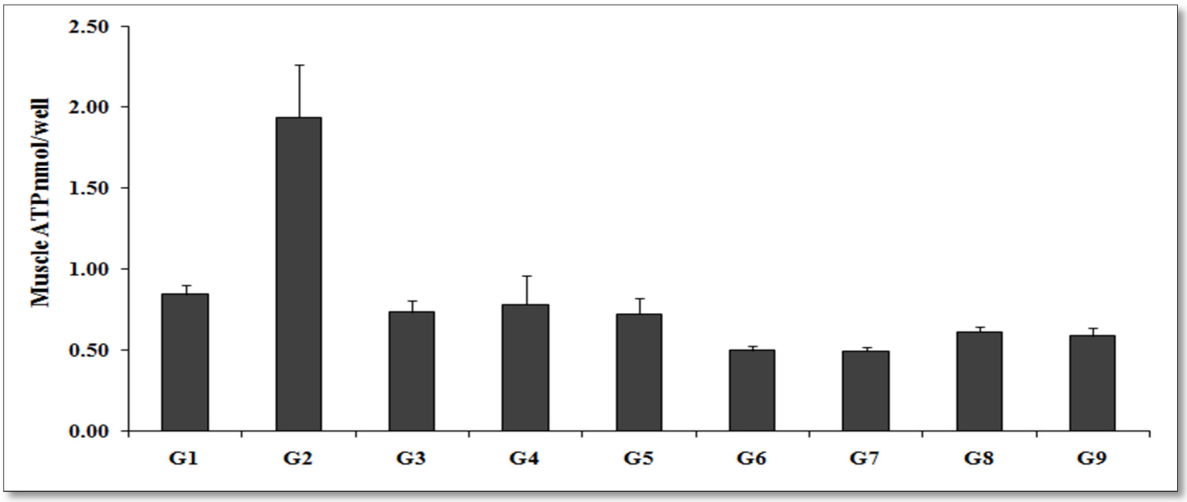

Figure 3. The effect of the test formulation on the level of muscle ATP in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + 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).

Estimation of Stomach Serotonin

Stomach and intestine are the primary reservoir of serotonin; it helps in controlling the bowel movements, function, regulate anxiety, happiness, and mood. Stress or stressful life events had a significant effect on gastrointestinal disease such as chronic disorders of the digestive system like inflammatory bowel disease (IBD), gastro-oesophageal reflux disease (GERD), gastrointestinal disorders (FGD), and peptic ulcer disease (PUD) [43]. Serotonin, biogenic amine [5-hydroxytryptamine (5-HT)] functions as a neurotransmitter play a vital role in stress conditions [44]. Stomach serotonin level in the unpredictable chronic stress (G2) was 2.67 ± 0.4 pg/mL, which was decreased by 34.9% as compared with the normal control (G1, 4.10 ± 0.6 pg/mL). Imipramine treatment (G3) showed an increased stomach serotonin level (3.49 ± 0.6 pg/mL) by 31% as compared to the G2. The untreated test formulation to the untreated rats (G4) decreased stomach serotonin level (2.12 ± 0.3 pg/mL) by 20.5% as compared with the G2. G5 group animals showed an increased stomach serotonin level (2.77 ± 0.4 pg/mL) by 4% and 30.8% as compared to the G2 and G4 groups, respectively. G6 group showed an increased stomach serotonin level (3.07 ± 0.3 pg/mL) by 15.1% and 44.8% as compared to the G2 and G4 groups, respectively. G7 group animals showed a significant increased stomach serotonin level (4.05 ± 0.8 pg/mL) by 51.9% and 91.2% ($p \leq 0.05$) as compared to the G2 and G4 groups, respectively. G8 group animals showed decreased stomach serotonin level (2.03 ± 0.3 pg/mL) as compared to G2 and G4. G9 group animals showed an increased stomach serotonin level (2.89 ± 0.6 pg/mL) by 8.3% and 36.3% as compared to the G2 and G4 groups, respectively **(Figure 4)**.

Estimation of Muscle Mitochondrial Assay - Citrate Synthase Activity

Mitochondria, play a major function in production of energy in cellular survival and death. Damage-associated molecular patterns (DAMPs) have been reported as a vital source in mitochondria. However, stress and its related factors causes major oxidative stress and inflammation that have been associated with cellular damage and citrate synthase activity, which is a main source of mitochondrial dysfunction [45]. The effect of the test formulation and Biofield Energy Treatment per se was estimated using the level of citrate synthase activity; the results are graphically presented in the **Figure 5**. Citrate Synthase activity in the unpredictable chronic stress (G2) was 3.63 ± 0.08 µunits/µL, which was decreased by 2.1% as compared to the normal control (G1, 3.71 ± 0.19 µunits/µL) group (G1). Imipramine treatment (G3) showed 3.27 ± 0.09 µunits/µL reduced level by 9.9% as compared to the G2. G4 group showed reduced value by 2.9% (3.52 ± 0.08 µunits/µL) as compared with the G2. However, group G5, G6, G7, G8, and G9 showed slight reduced value of Citrate Synthase level by 3.6%, 8.8%, 4%, 4.4%, and 2%, respectively as compared with the G2. Similarly, G5, G6, G7, G8, and G9 showed slight reduced value of Citrate Synthase level by 0.7%, 6.1%, 1.2%, 1.6%, and 0.9% respectively, as compared with the G4.

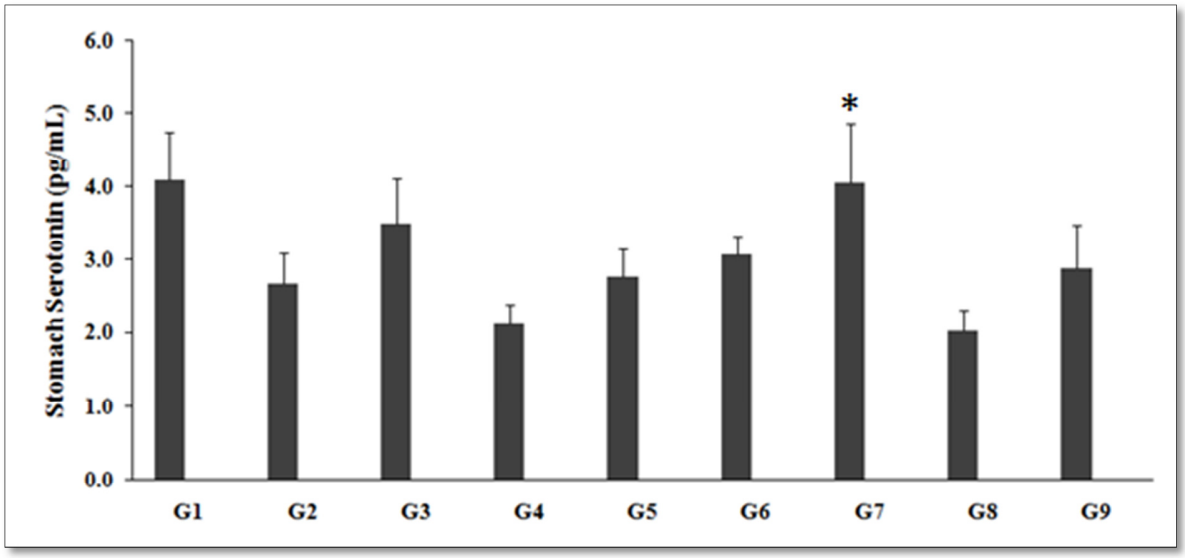

Figure 4. The effect of the test formulation on the level of stomach serotonin in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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.

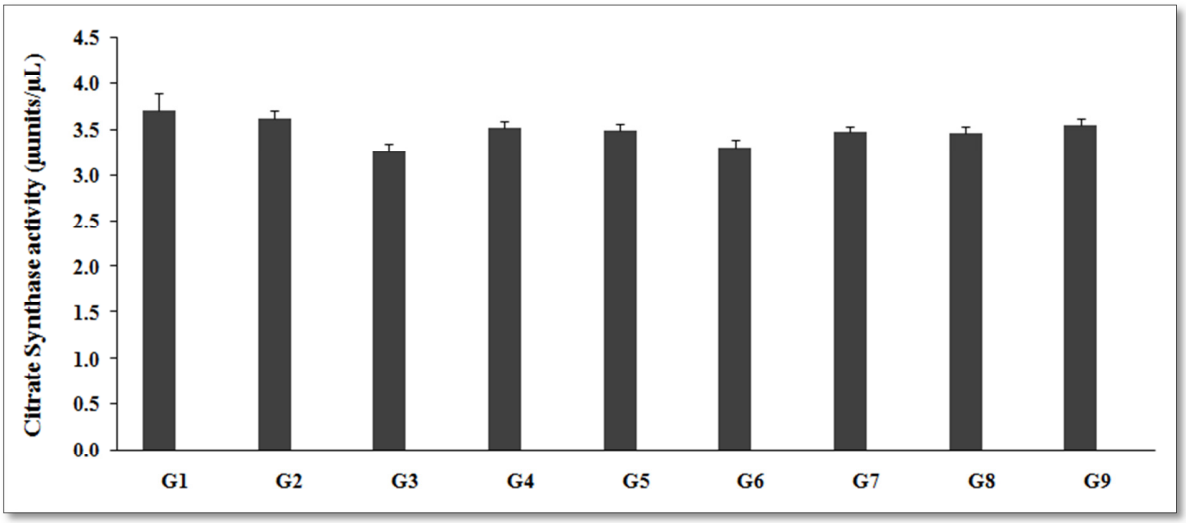

Figure 5. The effect of the test formulation on the level of citrate synthase activity in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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).

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-related all other symptoms/complications and also reduced the chances of disease susceptibility in these groups. Based on the overall data, it suggests that the Biofield Energy Healing/Blessing 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, slowdown 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

The unpredictable chronic stress (UCS) animal model was tested experimental for estimation of energy biomarkers with special reference to ATP level in brain, muscle, and liver along with level of stomach serotonin and mitochondrial citrate synthase activity. The level of ATP in brain was estimated and compared with respect to untreated test formulation and other preventive measure groups and was altered. The ATP level in liver was increased in the G7 and G9 groups, respectively as compared with the G4. Additionally, the ATP levels in the muscles was altered in all the treatment groups as compared to the G2 group. In addition to, stomach serotonin level was significantly increased by 30.8%, 44.8%, 91.2%, and 36.3% in the G5, G6, G7, and G9 groups, respectively as compared with the G4. Further, the expression of mitochondrial enzyme - citrate synthase in all the treatment groups was altered as compared to the both G2 and G4 groups. Thus, Biofield Energy Healing Treatment (the Trivedi Effect®) per se and other preventive maintenance groups (G7, G8, and G9) showed outstanding results in rat model study. The energy biomarkers were significantly improved after Biofield Energy Treatment and could be beneficial for low metabolic energy disorders like Niemann-Pick disease, Tay-Sachs disease, Gaucher disease, galactosemia, etc. It also helped to slowdown 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/Blessing 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 acute/chronic disease (auto-immune related and inflammatory disorders) progression rate and can be used in both before and after the manifestation of any disease symptoms in healthy, unhealthy, and ill peoples such as many thyroid disorders such hyperthyroidism, goiter, thyroid cancer, Hashimoto's thyroiditis, etc. This test formulation also can be used against fibromyalgia, Addison disease, multiple sclerosis, myasthenia gravis, aplastic anemia, psoriasis, rheumatoid arthritis, Crohn's disease, vitiligo, chronic fatigue syndrome 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 transient ischemic attack (TIA), and in the improvement of overall health and quality of life.

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