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In Vitro Evaluation of Anti-Rheumatoid Arthritis Potential of Biofield Energy Treatment by Inhibition of IL-8 Secretion Using Synovial Sarcoma Cell Line (SW982)

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Abstract

Interleukin-8 (IL-8) has proven a significant role in the inflammatory rheumatic disorder, which is produced in the synovial fluid. The objective of this study was to investigate the effect of Consciousness Energy Healing based DMEM medium in SW982 (Synovial cell line) on bone health inflammatory parameter like interleukin-8 (IL-8). The test sample (DMEM medium) was divided into three parts, first part received one-time Biofield Treatment by a renowned Consciousness Energy Healer, Alice Branton and was labeled as the one-time Biofield Energy Treated (BT-I) DMEM, while second part received the two-times the Biofield Energy Treatment and is denoted as BT-II DMEM. The third part did not receive any treatment and defined as the untreated DMEM group. Cell viability using MTT assay showed that the cell viability of SW982 cells was more than 95% with a safe and non-toxic profile. Besides, the two-times Biofield Treated DMEM group (BT-II) showed a significant ($p \leq 0.001$) inhibition of IL-8 by 31.82% as compared to the untreated DMEM group under stimulation with IL-1β in SW982 cells. Thus, data reflected the significant potential of the test items in bone-health, which play a vital role in the promotion and maintenance of strong and healthy bones against rheumatoid arthritis. Overall, Consciousness Energy Healing Treatment demonstrated a significant inhibition of pro-inflammatory cytokine (IL-8) that signifies it's potential to act as an anti-rheumatic action in arthritis and other bone inflammatory disorders along with autoimmune disorders such as osteoporosis, Paget's disease of bone, rickets, deformed bones, osteomalacia, osteoma, aging, bone loss, and fractures.

Keywords: Biofield Energy, Interleukin-8, Rheumatoid arthritis, DMEM, Synovial cell line, Bone Health

Introduction

Inflammation plays an important role in affecting the bone-health. It can interfere with the body's own natural ability to repair bone mass. Many of the bone disorders are mediated by inflammation as the common crucial factor. Rheumatoid arthritis (RA) is one of the common chronic autoimmune disorder categorized by systemic inflammation, persistent synovitis, and auto-antibodies. This inflammation of the joints leading to erosion of bone, prolonged deformities, and dysfunctional in the joints[1]. About 1% of the worldwide population is affected and most commonly are the middle-aged women. The chronic inflammation mainly of the small joints and synovial fluid leads to damage of the juxta-articular bone and articular cartilage[2]. As an autoimmune disease, its auto antigen has a wide range which would affect the specific immunomodulation and hampers the effective therapeutic approach. Increased secretion of inflammatory cytokines is a hallmark feature of RA. The transcriptional factors nuclear factor (NF)–kB and activator protein (AP)–1 are known to play an important role in gene regulation during inflammatory reactions, which regulates the transcription of inflammatory cytokines (interleukins), MMPs, and COX-2. Various pro-inflammatory cytokines have been reported with desirable roles such as synovial fluid interleukin-8 (IL-8) and neutrophil in RA[3].

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Besides, various chemo tactic factors released in the affected area are related to the employment and activation of leukocytes at the inflammation sites. Time course of leukocyte immigration and kind of cellular infiltration were determined by various triggering factors[4]. IL-8, a chemo tactic cytokine that was found to have a high selectivity for neutrophils formed by the mononuclear phagocytes and different tissue cells, on stimulation with interleukin-1 (IL-1), tumor necrosis factor (TNF), and bacterial endotoxins[5]. The SW982 (human synovial sarcoma cell line) cell is characterized by expression of inflammatory cytokines and matrix metalloproteinase (MMP) genes. The SW982 cells express genes encoding IL-1β, IL-6, cyclooxygenase (COX-2), and MMPs[6,7]. Hence, in the present study, inhibition of cytokine secretion (IL-8) by SW982 cells against IL-1β stimulated levels suggested beneficial effects of Biofield Energy Treatment in DMEM against RA.

Biofield Therapy has been reported as one of the significant CAM (Complementary and Alternative Medicine) approach, which was reported in many scientific studies with outstanding results and alterations in living and non-living materials[8]. Human has the ability to harness energy from the universal and can transmit it to any living organism(s) or nonliving object(s) around the globe. Biofield Energy Healing has been considered as Energy therapy, and was accepted by the National Center for Complementary and Alternative Medicine (NCCAM) [9]. NCCAM recommend and accepted various types of Energy therapies under CAM due to several advantages in addition to other treatments, medicines and practices such as natural products, Qi Gong, deep breathing, yoga, Tai Chi, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, acupressure, acupuncture, relaxation techniques, hypnotherapy, guided imagery, healing touch, movement therapy, Rolfing structural integration, mindfulness, Pilates, Reiki, Ayurvedic medicine, essential oils, traditional Chinese herbs and medicines, naturopathy, cranial sacral therapy, aromatherapy, and applied prayer (as is common in all religions, like Christianity, Hinduism, Buddhism and Judaism) [10,11]. Biofield Energy Healing Treatment (The Trivedi Effect®) contains putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed significant results in biological studies[12]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies[13]. The Trivedi Effect®- Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers[14-16], improved agricultural crop yield, productivity, and quality[17,18], transformed antimicrobial characteristics[19-21], biotechnology[22,23], improved bioavailability[24-26], skin health[27,28], nutraceuticals[29,30], cancer research[31,32], bone health[33-35], human health and wellness.

From the significant outcomes of Biofield Treatment in numerous previous studies, authors intend to evaluate the impact of the Biofield Energy Healing Treatment (The Trivedi Effect®) on DMEM as test sample for bone health activity with respect to the assessment of bone health parameter, inhibition of cytokine secretion (IL-8) against IL-1β stimulation using standard *in vitro* assaying SW982 cells.

Material and Methods

Chemicals and Reagents

Fetal Bovine Serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Ethylene Diaminetetra Acetic acid (EDTA), antibiotics solution like 'Penicillin-Streptomycin' was obtained from HiMedia, India however, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), and Direct Red 80 were purchased from Sigma, USA.

Cell Culture

The SW982 (Synovial cell line) was used as a test system in the present study. The SW982 cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37°C, 5% CO_2, and 95% humidity and sub cultured by trypsinization followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days before the start of the experiment, the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal-dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin[36].

Experimental Design

The experimental groups consisted of group 1 (G-I) with cells with the untreated DMEM. Group 2 (G-II) included one-time Biofield Energy Treated DMEM (BT-I) and group 3 (G-III) included the test item with two-times Biofield Energy Healing Treatment and denoted as BT-II.

Consciousness Energy Healing Treatment Strategies

The test sample (DMEM medium) was divided into three parts, first part received one-time Biofield Treatment by a renowned Consciousness Energy Healer, Alice Branton and was labeled as the one-time Biofield Energy Treated (BT-I) DMEM, while second part received the two-times the Biofield Energy Treatment and is denoted as BT-II DMEM. The third part did not receive any treatment and defined as the untreated DMEM groupA renowned Biofield Energy Healer, Alice Branton was provided this Biofield Energy Healing Treatment for ~5 minutes to the test items through the Healer's unique Energy Transmission process. The Biofield Energy Healer was located in the USA, while the test items were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Alice Branton in this study never visited the laboratory in person, nor had any contact with the test items. Further, the untreated DMEM group was treated with a "sham" healer for better comparative purposes. The "sham healer" did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

MTT Assay

MTT assay was used for the evaluation of cell viability in SW982 cells of the untreated and Biofield Energy Treated test items (DMEM). The details methodology of cell viability assay was followed by Lorraine et al. (2018) with few alterations[37]. The cytotoxicity of each tested concentration of the test items

was calculated with the help of Equation (1):

% Cytotoxicity = {(1-X)/R}*100…… (1)

Where, X = Absorbance of the Biofield Treated DMEM group; R = Absorbance of the untreated DMEM group

The percentage of cell viability corresponding to each treatment group was calculated by Equation (2):

% Cell Viability = (100 - % Cytotoxicity)…… (2)

The concentration exhibiting ≥70% cell viability was defined as non-cytotoxic[38].

Assessment of IL-8 using ELISA

The SW982 cell suspension in DMEM medium containing 10% FBS were plated at a density of 0.1 X 10^6 cells/well/mL in 12-well plates. The cells were incubated in a CO_2 incubator for 24 hours at 37°C, 5% CO_2 and 95% humidity. The cells were sera starved by replacing the fresh DMEM medium with 1% FBS for 24 hours. After 24 hours of sera starvation, the medium was removed and treated with 900 µL of the test items to each well along with inflammatory stimulus IL-1β at a final concentration of 0.25 ng/mL. After treatments, cells were further incubated in a 5% CO_2 incubator for 24 hours. The level of a cytokine (IL-8) in culture supernatants of SW982 cells was determined using ELISA as per manufacturer's instructions. The absorbance of each well was read at 450 nm using Synergy HT microplate reader, BioTek, USA.

Statistical Analysis

All the values were represented as the mean ± standard error of the mean (SEM). For multiple groups comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Cell Viability Study Using MTT Assay

The percentage of cell viability in SW982 cell using MTT assay was performed among the Biofield Energy Treated test sample (DMEM medium). The data in term of percentage values are presented in Figure 1. The percentage of cell viability showed a significantly improved cell viability in the test items groups. The results showed that the test sample was found to have significant cell viability with more than 95%. The one-time Biofield Energy Treated (BT-I) group showed 95.8% and two-times Biofield Energy Treated (BT-II) group showed 104.6% cell viability (Figure 1). Overall, experimental MTT assay data suggested that the Biofield Energy Treated DMEM was found safe and non-toxic. Thus, the test sample was used to study the bone health parameter, cytokine IL-8 in SW982 cells.

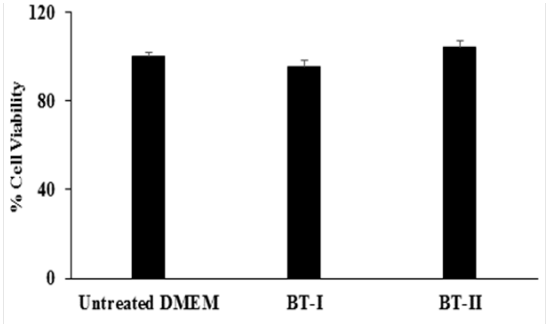

Figure 1: Assessment of cell viability using MTT assay of the test items (DMEM) on SW982 cells. BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM

Effect of the Test Items on IL-8 in SW982 cells

The effect of the test items on the secretion of IL-8 in SW982 cells against IL-1β stimulation was determined after 24 hours. Figure 1 demonstrated the level of IL-8 in SW982 cells. The untreated DMEM group showed 534.6 ± 13.32 pg/mL of IL-8. Besides, the level of IL-8 was significantly ($p \leq 0.001$) inhibited by 31.82% in the two-times Biofield Energy Treated DMEM (BT-II) group as compared to the untreated DMEM group; while 31.16% inhibited as compared to the one-time Biofield Energy Treated (BT-I) group after stimulation with the IL-1β in SW982 cells (Figure 2).

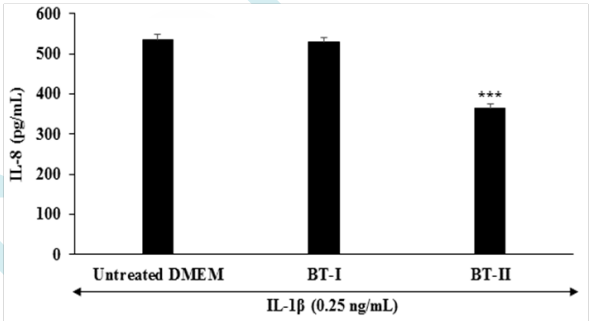

Figure 2: Effect of test item on the secretion of IL-8 in SW982 cells after stimulated with IL-1β.BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. ***$p \leq 0.001$ *vs.* untreated DMEM group.

The scientific studies showed the importance of inflammation in the synovial membrane, proliferation in its lining are one of the major changes occurred in rheumatoid arthritis (RA). These inflammation results in significant erosion of the cartilage and bone. Macrophage-like synoviocytes and the fibroblast-like synoviocytes play a serious role in the joint destructive process[39]. The major mediators of joint inflammations are the IL-6 and IL-8[40,41]. Pro-inflammatory mediator's expression was promoted by the IL-1β produced by the synovial lining macrophages[42]. Thus, the mechanisms of IL-8 and to reduce its expression are one the best approach against any joint inflammation disorders such as RA. Besides, the experimental model using SW982 cells demonstrated significant inhibition of IL-1β stimulated IL-8 secretion in SW982 cells by the test items, which would play a significant role in the bone-health and work as a best supplementation to treat various bone and age-related diseases such as osteoporosis. Overall, the data suggested that The Trivedi Effect®-Energy of Consciousness Healing based

DMEM could be used to improve the bone health and worked as an anti-RA activity in bone health.

Conclusions

MTT assay for cell viability showed significant improved cell viability with more than 95.8% among all the tested groups, which suggested that the test items were safe and nontoxic. In addition to, the effect of the two-times Biofield Energy Treated DMEM (BT-II) on IL-1β stimulated secretion of IL-8 in SW982 cells after 24 hours demonstrated a significant inhibition of IL-8 level by 31.82% as compared to the untreated DMEM group. Thus, the Biofield Energy Treated (The Trivedi Effect®) DMEM was found to have significant impact on rheumatoid arthritis, which is very vital to combat against various bone-related disorders. Thus, with respect to the untreated DMEM, Biofield Energy Treated DMEM would be highly significant in the growth of SW982 cells on bone health. Therefore, the Consciousness Energy Healing based DMEM might be a suitable alternative media for cell growth. It can be useful for the management of rheumatoid arthritis and against various bone-related disorders such asosteoma, osteoporosis, Paget's disease of bone, osteomalacia, rickets, deformed bones, etc. Apart from it could be useful for the management of hormonal balance, stress, aging, and other bone disorders that occurs due to poor nutrition, genetically predefined, etc. Besides, it might be useful various immune-related disease conditions such as Sjogren Syndrome, Ulcerative Colitis, Dermatomyositis, Alzheimer's Disease, Myasthenia Gravis, Dermatitis, Irritable Bowel Syndrome, Graves' Disease, Asthma, Hashimoto Thyroiditis, Pernicious Anemia, Multiple Sclerosis,Systemic Lupus Erythematosus, Aplastic Anemia, Hepatitis, Atherosclerosis, Diverticulitis, Diabetes, Parkinson's Disease, etc.

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Abbreviations

CAM: Complementary and Alternative Medicine; NCCAM: National Center for Complementary and Alternative Medicine; DMEM: Dulbecco's Modified Eagle's Medium; FBS: Fetal Bovine Serum.

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