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An Alternative Therapeutic Approach on Bone Cells Differentiation after Administration of Biofield Energy Treated Vitamin D₃ in MG-63 Cell line

Abstract

The objective of the current experiment was to evaluate the potential of Consciousness Energy Healing-based test items (Vitamin D3 and DMEM) on bone parameters. Both the TI were distributed into two individual parts. One part of each samples was received Consciousness Energy Healing Treatment by Eileen Mary Meagher and those samples were labelled as the Biofield Treated (BT) samples, while the other parts of each samples were denoted as the untreated test items (UT). Cell viability data showed more than 71% viable cells signifies a safe and nontoxic profile of test items on MG-63 cell line. ALP was significantly increased by 132.18% and 126.52% in BT-DMEM+UT-TI and BT-DMEM+BT-TI, respectively at 50 µg/mL; while 139.06% (at 0.1 µg/mL) in UT-DMEM+BT-TI compared to untreated. Collagen was significantly increased by 172.76% at 1 µg/mL in UT-DMEM+BT-TI, while 89.65% in BT-DMEM+UT-TI at 10 µg/mL than untreated. Additionally, collagen was significantly increased by 111.2% in BT-DMEM+UT-TI at 50 µg/mL, while 113.16% in BT-DMEM+BT-TI at 100 µg/mL than untreated. The percent of bone mineralization was significantly increased by 206.21%, 168.74%, and 224.82% in UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI, respectively at 0.1 µg/mL compared with the untreated. In addition, percent of bone mineralization data showed a significant increase by 190.63%, 181.07%, and 245.41% in UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI, respectively at 1 µg/mL than untreated. Moreover, at 10 µg/mL it was significantly increased by 129.44%, 111.76%, and 109.81% in UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI, respectively at 10 µg/mL with respect to untreated. Based on the study outcomes it emphasized that Biofield Energy Treatment could regulate the osteoblast function, improves bone mineralization, and calcium absorption in wide range of bone disorders along with wide range of autoimmune diseases.

Keywords: Energy healing; The Trivedi effect®; Osteosarcoma cells; Vitamin D; Bone disorders; Bone mineralization

Abbreviations

ALP: Alkaline Phosphatase; FBS: Fetal Bovine Serum; CAM: Complementary and Alternative Medicine; NCCAM: National Center for Complementary and Alternative Medicine; MG-63: Human Bone Osteosarcoma Cells, DMEM: Dulbecco's Modified Eagle's Medium; EDTA: Ethylene Diamine Tetra Acetic Acid; UT: Untreated; BT: Biofield Energy Treated; TI: Test Item

Introduction

Bone-related health issues become a major problem among the population from village to the cities. Vitamin D plays an important role to keep a healthy mineralized skeleton of most of the vertebrates



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including humans. It also used against aging, inflammation and improved overall quality of life. Naturally, it is synthesized in the skin with the help of sunlight after-that, it is metabolized in the liver and kidney to form the active metabolite 1,25-dihydroxyvitamin D (calcitriol) [1,2]. Calcitriol play an important role in maintaining the normal level of calcium and phosphorus, promotes bone mineralization, induce or repress the genes responsible for conserving the mineral homeostasis and skeletal integrity [3]. Various *in vitro* study data demonstrated about three important key biomarkers, such as alkaline phosphatase (ALP), collagen for bone cells growth and development. MG-63 cells were obtained from juxtacortical osteosarcoma, which is an immature osteoblast phenotype and undergoes temporal development in long-term culture. The response of MG-63 cells to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) administration has been studied to be similar to normal human osteoblast cells [4]. Hence, MG-63 cell line is widely used for studying the potential of any test compounds to improve the bone health [5]. Vitamin D has diversified effects which regulate the functions in different organs such as brain, liver, lungs, kidneys, etc. Additionally, it has significant anti-aging, anti-inflammatory, anti-stress, and anti-cancer. Vitamin D receptors (VDRs) are widely present in most of the vital body organs such as heart, brain, lungs, kidney, liver, etc. and responsible for cell-to-cell communication, proliferation, neurotransmission, immune and cardiovascular functions, etc. [1]. Therefore, authors evaluated the *in vitro* effect of the Biofield Treated vitamin D₃ as a test item, a Complementary and Alternative Medicine (CAM) on bone health using MG-63 cell line for major biomarkers.

The effects of the CAM therapies have great potential, which include Johrei, external qigong, Qi Gong, Reiki, therapeutic touch, deep breathing, yoga, polarity therapy, Tai Chi, pranic healing, chiropractic/osteopathic manipulation, acupressure, acupuncture, guided imagery, special diets, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, Roling structural integration, relaxation techniques, Ayurvedic medicine, movement therapy, healing touch, pilates, mindfulness, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [6]. Biofield

Energy Healing Treatment (The Trivedi Effect) contain a putative bioenergy, which is channeled by a renowned practitioners from a distance. Biofield Energy Healing as a CAM showed a significant results in biological studies [7]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies [8]. The Trivedi Effect-Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers, improved agricultural crop yield, productivity, and quality, transformed antimicrobial characteristics, improved bioavailability, biotechnology, skin health, nutraceuticals, cancer research, and human health and wellness [9-27]. Thus, based on the above facts and importance authors planned this experiment to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect) on vitamin D₃ for bone health activity with respect to ALP, collagen content, and bone mineralization in MG-63 cells.

Material and Methods

Chemicals and reagents

Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India, while 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium (MTT), Direct Red 80, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, USA. Rutin was purchased from TCI, Japan, while vitamin D₃ and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Rest-over chemicals used in this study were analytical grade obtained from India.

Cell culture

In the present study human bone osteosarcoma cell line (MG-63) was used as the test system. The MG-63 cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37 °C, 5% CO₂ and 95% humidity and sub-cultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days prior to experiment (i.e. Day 3), 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 [4].

Study design

To perform this experiment the following groups were allocated. These are consisting of cells in baseline control (untreated cells group), vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), positive control group (rutin) and experimental test groups. The experimental test groups included the combination of the Biofield Energy Treated (BT) and untreated (UT) vitamin D₃/DMEM. It consisted of four treatment groups on specified cells with UT-DMEM+Untreated-Test item (UT-TI), UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI.

Preparation of test items

Vitamin D₃ was weighed and dissolved in suitable solvent at 10 mM-50 mM (based on the requirement of the assay). Stock solution

was further diluted in SFM to treat cells. Besides, commercially supplied DMEM was dissolved in 800 mL of distilled water. Added calculated amount of NaHCO₃, adjusted pH (7.2-7.4), and 10 mL of penicillin/streptomycin were added to make final volume 1 L. Then, filtered into sterile flasks using 0.2 µm filter using peristaltic pump and checked for sterility by incubating in a CO₂ incubator for 24 hours. Then, stored the content at 2-8 °C till used. Here, concentration (µg/mL) of vitamin D₃ was used as specific amount and mixed in DMEM during experiment.

Biofield energy treatment strategies

The test item and DMEM were divided into two parts. One part each of the test item and DMEM was treated with the Biofield Energy (The Trivedi Effect) by a renowned Biofield Energy Healer and coded as the Biofield Energy Treated item, while the second part did not receive any sort of treatment. However, the untreated group was treated with a "sham healer" for well comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. This Biofield Energy Healing Treatment was provided by Eileen Mary Meagher remotely for ~5 minutes through the Healer's unique Energy Transmission process remotely to the test samples under laboratory conditions. Biofield Energy Healer was remotely located in the USA, while the test samples were located in the reputed research laboratory (Dabur Research Foundation), New Delhi, India. Healer, neither visited to the laboratory in person, nor had any contact with the test items. After that, the untreated and Biofield Energy Treated test samples were kept in proper storage conditions until end of the study.

MTT Assay

The cell viability was performed using MTT assay in MG-63 cells. The details procedure of cell viability assay was followed by Allen KB et al. with slight modification [28]. The cytotoxicity of each tested concentration of the test items was calculated with the help of Equation (1):

$$\% \text{ Cytotoxicity} = \left\{ \frac{1 - X}{R} \right\} * 100 \dots \dots \dots (1)$$

Where,

X=Absorbance of treated cells;

R=Absorbance of untreated cells

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

$$\% \text{ Cell Viability} = (100 - \% \text{ Cytotoxicity}) \dots \dots \dots (2)$$

The concentration exhibiting ≥70% cell viability was appraise as non-cytotoxic.

Alkaline Phosphatase (ALP) activity

The effect of the Biofield Energy Treatment on the test items for the evaluation of ALP activity in MG-63 cells. The procedure of cell counting, plating, and treatment was followed as per Liu SC et al. [30]. The percent increase in ALP activity with respect to the untreated

cells was calculated using Equation (3):

$$\% \text{ Increase in ALP} = \left\{ \frac{X - R}{R} \right\} * 100 \dots \dots \dots (3)$$

Where,

X = Absorbance of cells corresponding to positive control and test groups;

R = Absorbance of cells corresponding to untreated cells

Collagen activity

The MG-63 cells were used for the evaluation of the potential of Biofield Treated test items and the procedure in details was as per Parulkar VR et al. with few modifications [30]. The increase collagen level with respect to the untreated cells was calculated using Equation (4):

$$\% \text{ Increase in collagen levels} = \left\{ \frac{X - R}{R} \right\} * 100 \dots \dots \dots (4)$$

Where,

X = Collagen levels in cells corresponding to positive control and test groups;

R = Collagen levels in cells corresponding to untreated cells

Bone mineralization activity

Evaluation of the percent increased of mineralization after treatment of the Biofield Treated test items in MG-63 cells, and the details steps were followed according to Slade TC et al. [31]. The percentage increase in bone mineralization compared to the untreated cells was calculated using Equation (5):

$$\% \text{ Increase} = \left\{ \frac{X - R}{R} \right\} * 100 \dots \dots \dots (5)$$

Where,

X = Absorbance in cells corresponding to positive control or test groups;

R = Absorbance in cells corresponding to untreated group

Statistical analysis

Data were represented as percentage of respective parameters. For multiple group 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≤0.05.

Results and Discussion

Cell viability study using MTT

The MTT assay is one of the preliminary method of biological evaluation and screening of cytotoxicity of the test samples. In this assay, using cells in order to check the growth, proliferation, its reproduction and morphological effects in presence of test samples. All the test samples were initially screened for cell viability assay in MG-63 cells. The percent cell viability results are graphically shown in Figure 1. MTT assay data showed that all the tested samples at various

concentrations along with positive control, rutin were found to have significant cell viability with more than 71%. In addition, Biofield Energy Healing Treatment on test samples showed significant improved cell viability in different tested groups. Overall, the test data suggests that the test item along with DMEM groups were found safe at all the tested concentrations range up to maximum of 100 µg/mL against the tested MG-63 cells and was tested for various bone health parameters such as on the levels of alkaline phosphatase (ALP) activity, collagen synthesis, and bone mineralization.

Alkaline Phosphatase (ALP) enzyme activity

Total ALP activity in serum has been commonly used as a biochemical marker of osteoblast function. However, it has lacks of specificity because of the contribution of activity derived from the liver, in particular. Human alkaline phosphatases (ALP) are a group of enzymes of similar specificity coded for by at least four different gene loci that catalyze the hydrolysis of phosphate esters at an alkaline pH [32,33]. In the growth phase of bone differentiation, the level of ALP became increased. However, abnormal level of ALP bone isoenzyme is reported in various pathological conditions, viz. osteoporosis, healing fracture, bone growth, acromegaly, myelofibrosis etc. The level can be improved using supplementation of vitamin D, calcium and ALP enzymes [33-35]. The test samples were screen for the level of ALP in different groups at various concentrations on MG-63 cell line (Figure 2). The results in terms of percentage ALP were described and compared with respect to the untreated group. The positive control, rutin showed a significant increased values of ALP by 33.97%, 45.69%, and 79.66% at 0.01, 0.1 and 1 µg/mL respectively.

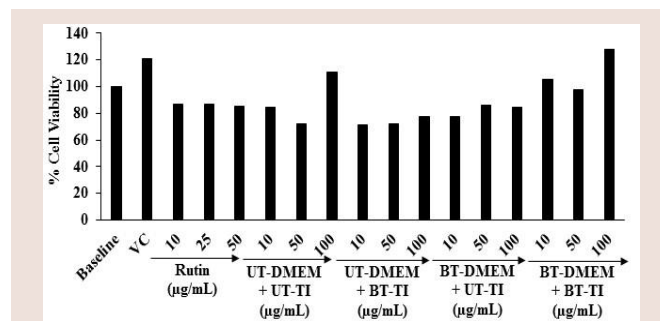


Figure 1: The MTT assay of the test items on MG-63 cell line after 72 hours of treatment. VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Energy Treated; TI: Test Item.

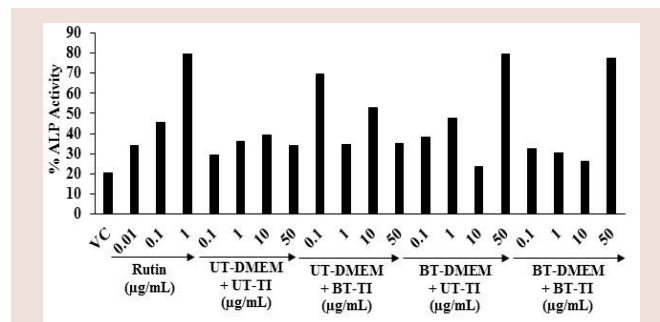


Figure 2: Estimation of alkaline phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line. VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Energy Treated; TI: Test Item.

The experimental data showed that the ALP level was significantly increased by 139.06%, 30.43% and 11.26% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 0.1 µg/mL as compared with the untreated test item and DMEM group. However, the level of ALP was significantly increased by 32.81% in the BT-DMEM+UT-TI group at 1 µg/mL as compared with the untreated test item and DMEM group. Further, ALP level was significantly increased by 25.74% in the UT-DMEM+BT-TI group at 10 µg/mL, respectively as compared to the untreated test item and DMEM group. However, at 50 µg/mL the level of ALP was significantly enhanced by 2.25%, 132.18% and 126.52% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively as compared to the untreated test item and DMEM group. Overall data concluded that The Trivedi Effect[®]-Energy of Consciousness Healing based vitamin D₃ and DMEM could be used to improve the ALP concentration in many bone disorders [34]. In conclusion, the experimental data suggest that Biofield Energy Healing Treatment in test samples showed a significant growth of bone health that can be used as supplementation against many bone related diseases.

Collagen synthesis

Collagen is one of the vital protein of extracellular matrix part of the body having a potential utility in the treatment of various bone-related disorders like osteoarthritis and osteoporosis. The results of collagen activity expressed as percentage with respect to the untreated cells group is shown in Figure 3. The reference item (rutin hydrate) showed a significant increased collagen by 7.14%, 24% and 50.29% at 0.001, 0.01 and 0.1 µg/mL, respectively. Besides, the experimental test group UT-DMEM+BT-TI showed a significant increased collagen level by 172.76% at 1 µg/mL as compared with the untreated test item and DMEM group. Additionally, collagen synthesis was significantly increased by 34.48% and 89.65% in the BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 10 µg/mL, as compared to the untreated test item and DMEM group. Collagen level was desirably increased by 46.53% and 111.2% in the BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 50 µg/mL, as compared to the untreated test item and DMEM group. However, at 100 µg/mL, the level of collagen synthesis was significantly increased by 113.16% in the BT-DMEM+BT-TI group as compared to the untreated test item and DMEM group. According to Titorencu et al. reported that vitamin D₃ (1, 25 dihydroxycholecalciferol) one of the active

metabolite of vitamin D (cholecalciferol) can scaffolds with collagens and ultimately sustained the proliferation of human osteoblast cells [36,37]. Therefore, this types of collagen-vitamin D₃ scaffolds have been used in various medical applications such as drug delivery, soft tissue augmentation, hemostatic pads, skin substitutes, suturing and as tissue engineering substrate [38,39]. Overall, the present outcomes suggested that the Biofield Energy (The Trivedi Effect[®]) Treated vitamin D₃ showed a significant improved the level of collagen that can be used to improve the bone health.

Bone mineralization

The bone mineralization activity was done in order to check the bone calcification, which plays an important role in the treatment of osteoporosis or other bone-related disorders. The results of bone mineralization activity are presented in term of percentage change among different experimental groups in Figure 4. The positive control, rutin group showed a significant increased value of bone mineralization by 68.93%, 84.60%, and 134.46% at 5, 10 and 25 µg/mL respectively. The bone mineralization activity was significantly enhanced by 206.21%, 168.74%, and 224.82% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 0.1 µg/mL as compared with the untreated test item and DMEM group. Moreover, the percentage of bone mineralization was significantly increased by 190.63%, 181.07%, and 245.41% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 1 µg/mL as compared with the untreated test item and DMEM group. Further, at 10 µg/mL the percentage of bone mineralization was significantly increased by 129.44%, 111.76%, and 109.81% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 10 µg/mL with respect to the untreated test item and DMEM group. The experimental findings showed that Biofield Energy Healing Treatment on test samples had significantly improved the rate of bone mineralization as compared with the untreated groups, which can be used in various bone-related disorders and recovery process. Improved bone mass after Biofield Energy Treatment results due to the improved bone mineralization capacity and increased calcium absorption in bones [40,41].

Conclusions

Cell viability using MTT assay with more than 71% among the tested groups, which suggest that test samples are found as safe and nontoxic. ALP level was increased by 132.18% and 126.52% in the

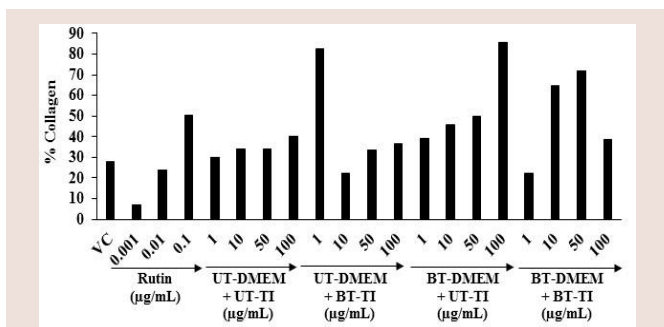


Figure 3: The effect of the test items on MG-63 cell line for collagen level. VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Energy Treated; TI: Test Item.

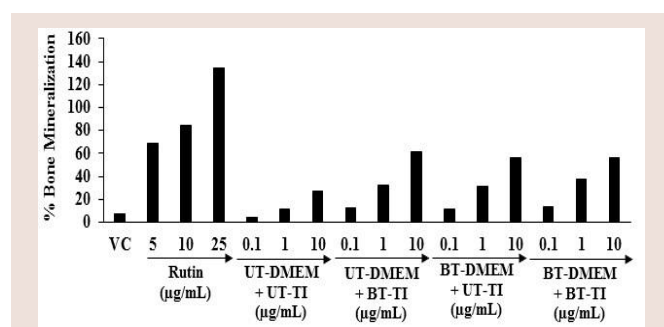


Figure 4: The effect of the test items on MG-63 cell line for bone mineralization. VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Energy Treated; TI: Test Item.

BT-DMEM+UT-TI and BT-DMEM+UT-TI groups, respectively at 50 µg/mL while 139.06% (at 0.1 µg/mL) in the UT-DMEM+BT-TI groups compared to the untreated test item and DMEM group. Collagen level was significantly increased by 34.48% and 89.65% in the BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 10 µg/mL; while 172.76% at 1 µg/mL in the UT-DMEM+BT-TI groups compared to the untreated test item and DMEM group. In addition, 46.53% and 111.2% increased the level of collagen in the BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 50 µg/mL, while 113.16% in the BT-DMEM+BT-TI group at 100 µg/mL compared to the untreated test item and DMEM group. Besides, the bone mineralization percent was significantly increased by 206.21%, 168.74%, and 224.82% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 0.1 µg/mL as compared with the untreated group. In addition, the percent of bone mineralization data showed a significant increased by 190.63%, 181.07%, and 245.41% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 1 µg/mL compared with the untreated group. Moreover, at 10 µg/mL the percentage of bone mineralization was significantly increased by 129.44%, 111.76%, and 109.81% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 10 µg/mL with respect to the untreated test item and DMEM group. Overall, the Biofield Energy Treated (The Trivedi Effect) test samples were found to have a significant impact on tested bone health parameters viz. collagen, bone mineralization, and ALP, which are very vital to combat the bone disorders. Therefore, the Consciousness Energy Healing-based vitamin D₃ might be a suitable nutritional supplement for the management of various bone-related disorders viz. rickets, osteoporosis, stress, aging, Paget's disease of bone, osteoma, osteomalacia, bone and/or joint pain, bone loss and fractures, hormonal imbalance, and other bone diseases that are caused by poor nutrition, genetics, or problems with the rate of bone growth or rebuilding. Further, Biofield Energy Treated vitamin D₃ can be useful as anti-aging, anti-inflammatory, anti-stress, anti-arthritis, anti-osteoporosis, anti-cancer, anti-apoptotic, roles. Besides, it can also be utilized in organ transplants like kidney, liver and heart transplants, hormonal imbalance, and various inflammatory conditions such as Alzheimer's Disease, Ulcerative Colitis, Dermatitis, Irritable Bowel Syndrome, Hashimoto Thyroiditis, and also useful against immune-related disorders like Diabetes, Parkinson's Disease, Aplastic Anemia, Pernicious Anemia, Multiple Sclerosis, Hepatitis, Graves' Disease, Myasthenia Gravis, Atherosclerosis, Systemic Lupus Erythematosus, etc. near future.

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