Pharmaceutical Sciences & Analytical Research Journal
ISSN: 2640-6659

Research Article *Volume 4 Issue 1*

Influence of the Consciousness Energy Healing Treatment on the Structural Properties and Isotopic Abundance Ratio of Magnesium Gluconate

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Received Date: January 20, 2021; **Published Date:** February 04, 2021

Abstract

Magnesium is a second most abundant mineral in the human body and, which is required for in more than 300 enzymatic reactions, normal functioning of body organs, etc. The main objective of the current study was to investigate the impact of The Trivedi Effect®- Consciousness Energy Healing Treatment on magnesium gluconate for the change in the isotopic abundance ratio (PM+1/PM and PM+2/PM) along with the structural properties using LC-MS and NMR spectroscopy. Magnesium gluconate sample was divided into two parts – one part termed as control, while the other part was treated with The Trivedi Effect®-Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi and termed as Biofield Energy Treated sample. The LC-MS analysis of the control and Biofield Energy Treated samples exhibited the presence of the molecular mass of magnesium gluconate adduct with hydrogen ion at *m/z* 415 (calcd for $C_{12}H_{23}MgO_{14}$+, 415.09) at the retention time of 1.67 minutes and almost similar fragmentation pattern. But, the relative peak intensities of the fragmented ions of the Biofield Energy Treated sample were significantly changed compared with the control sample. The proton and carbon signals for CH, CH_2, OH, and CO groups in the ^{1}H and ^{13}C NMR spectra of the treated sample were almost close compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{25}Mg/^{24}Mg$) in the treated magnesium gluconate was significantly decreased by 60.41% compared to the control sample. Therefore, the ^{13}C, ^{2}H, ^{17}O, and ^{25}Mg contributions from $(C_{12}H_{23}MgO_{14})$+ to *m/z* 416 in the treated sample was significantly decreased compared to the control sample. Similarly, the isotopic abundance ratio of P_{M+2}/P_M ($^{18}O/^{16}O$ or $^{26}Mg/^{24}Mg$) in the treated sample was significantly decreased by 79.33% compared to the control sample. Briefly, the ^{18}O and ^{26}Mg contributions from $(C_{12}H_{23}MgO_{14})$+ to *m/z* 417 in the treated magnesium gluconate significantly decreased compared with the control sample. Thus, The Trivedi Effect®-Consciousness Energy Healing Treated magnesium gluconate might be supportive to design the novel potent enzyme inhibitors and better pharmaceutical and nutraceutical formulations through its changed intrinsic properties. This Biofield Energy Treated magnesium gluconate could prove better therapeutic response against various diseases such as diabetes mellitus, aging, allergy, immunological disorders, inflammatory diseases, and other chronic infections.

Keywords: Magnesium gluconate; Biofield Energy; The Trivedi Effect®; Energy of Consciousness Healing Treatment; LC-MS; NMR; Isotopic abundance ratio; Isotope Effects

Trivedi MK and Jana S. Influence of the Consciousness Energy Healing Treatment on the Structural Properties and Isotopic Abundance Ratio of Magnesium Gluconate. Pharma Sci Analytical Res J 2021, 4(1): 180033.

Abbreviations: NIH/NCCAM: National Institute of Health/National Center for Complementary and Alternative Medicine; MS: Mass Spectrometry; GC: Gas Chromatography; LC: Liquid Chromatography; CAM: Complementary and Alternative Medicine; NMR: Nuclear Magnetic Resonance; PDA: Photo-diode Array; RT: Retention Time; TIC: Total Ion Chromatograms; PPM: Parts Per Million; ESI: Electro Spray Ionization.

Introduction

Magnesium gluconate is the best source of magnesium for the human body, which has a higher level of magnesium bioavailability than any other magnesium salt [1]. Several magnesium (Mg^{2+}) containing enzymes are found in the physiological systems and act as a cofactor in more than 300 enzymatic reactions [2,3]. Magnesium is a second most abundant mineral in the human body, and its deficiency may cause hypomagnesaemia, responsible for many chronic disorders like Alzheimer's, hypertension, stroke, etc [1-3]. Gluconic acid and its derivatives have widespread applications in the nutraceuticals and pharmaceutical industries [4]. Magnesium gluconate is a well-known powerful antioxidant, which used alone or in combination with other antioxidant for the treatment and prevention of various disease, i.e. diabetes mellitus, Alzheimer's disease, asthma, cardiovascular diseases, cancer, inflammatory diseases, immunological disorders, septic shock, pre-eclampsia, eclampsia, oxidative stress-induced ischemia/reperfusion injury, etc [4-8]. Magnesium deficiency in the body is mainly due to the poor diet, stomach/intestinal absorption problem, severe diarrhea/vomiting, and the adverse effect of drugs like furosemide and hydrochlorothiazide, etc [9], which can be overcome by a well-balanced diet and magnesium supplements [10]. Therefore, magnesium gluconate was considered as one of the components in the formulation as a source of magnesium for the prevention and treatment of various magnesium deficiency diseases.

The Trivedi Effect®-Consciousness Energy Healing Treatment has been known for its impact on the various properties of drug substances that would be helpful in the modification of physicochemical properties which enhance the druggable properties of pharmaceutical/nutraceutical compounds [11-13].

Biofield Energy is a kind of unique energy, i.e. the para-dimensional electromagnetic field in the human body, resulting the continuous emission of energy from the body, which can freely flow between the human and environment [14,15]. Since ancient times, different Hindu, Chinese, Japanese, etc. religions have recognized a living force that preserves and inhabits every living organism and believed to co-relate with the soul, spirit, and mind. Scientifically, this hypothetical vital living force has been evaluated and is considered as the Bioenergetics Field. There are several types of Biofield Energy Healing Therapies that are known for their significant impacts against various disease conditions [16]. Biofield Energy Healers possess the ability to harness the energy from the "Universal Energy Field" and can transmit this energy into any living or non-living object(s), which respond to useful way, and the process is called Biofield Energy Healing Treatment. Such type of energy therapies are recommended by the National Institute of Health/National Center for Complementary and Alternative Medicine (NIH/NCCAM), and they included them under the Complementary and Alternative Medicine (CAM) due to their several advantages [17].

In recent years remarkable, outstanding results of The Trivedi Effect®-Biofield Energy Treatment have been scientifically reported in the field of materials science [18-20], agriculture [21-23], biotechnology [24,25], microbiology [26,27], pharmaceutical sciences [12,13,28], and medical science [29-31]. As per the literature, The Trivedi Effect® also has the remarkable effect to alter the isotopic abundance ratios of various compounds; this might be through the possible mediation of neutrinos [31-33]. The natural stable isotope ratio analysis of an element has the wide applications in several scientific fields to understand the isotope effects resulting from the variation of the isotopic composition of that molecule [34-36].

Conventional mass spectrometry (MS) techniques such as gas chromatography – mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), are widely used for the analysis of isotope ratio with sufficient precision [35]. Therefore, LC-MS based isotopic abundance ratio analysis of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{25}Mg/^{24}Mg$) and P_{M+2}/P_M ($^{18}O/^{16}O$ or $^{26}Mg/^{24}Mg$) was performed to evaluate the influence of The Trivedi Effect® - Consciousness Energy Healing Treatment on the isotopic abundance ratio in magnesium gluconate. Consequently, in this study, the LC-MS and NMR (Nuclear Magnetic Resonance) techniques were also used to characterize the structural properties of the magnesium gluconate samples.

Materials and Methods

Chemicals and Reagents

Magnesium gluconate was purchased from Tokyo Chemical Industry Co., Ltd., Japan. All other chemicals used in the experiment were of analytical grade available in India.

Consciousness Energy Healing Treatment Strategies

The Magnesium gluconate test compound was equally divided into two parts. One part of magnesium gluconate was

termed as Biofield Energy Treated sample, which received the Energy of Consciousness Healing Treatment by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi (USA) remotely under the standard laboratory conditions for 3 minutes. Besides, the other part of magnesium gluconate was denoted as the control sample, which did not receive the Biofield Energy Treatment, but was subjected to "sham" healer under the similar laboratory conditions, who did not have any knowledge about the Biofield Energy Healing Treatment. Consequently, the control as well as Biofield Energy Treated magnesium gluconate samples were kept in similar sealed conditions and further analyzed by using LC-MS and NMR analytical techniques.

Characterization

Liquid chromatography-mass spectrometry (LC-MS) analysis and Calculation of Isotopic Abundance Ratio: The LC-MS analysis of the control and Biofield Energy Treated magnesium gluconate was carried out with the help of LC-Dionex Ultimate 3000, MS-TSQ Endura, USA equipped with a photo-diode array (PDA) detector connected with a triple-stage quadrupole mass spectrometer (Thermo Scientific TSQ Endura, USA) with a Thermo Scientific Ion Max NG source and heated electrospray ionization (ESI) probe. The analysis was performed on a reversed phase Zorbax SB-C18 100 × 4.6 mm, 3.5 µm in gradient mode in the liquid chromatograph. Mobile phase were 0.5% formic acid in water (mobile phase A), and 0.5% formic acid acetonitrile (mobile phase B) at a constant flow rate of 0.3 mL/min. The column temperature was kept constant at 35˚C. The injection volume was 10 µL and the total run time was 15 min. Chromatographic separation was achieved using gradient condition as follow: 0.1 min-3%B, 5.0 min-50%B, 8.0 min-95%B, 10 min-3%B, and 15 min-3%B. Peaks were monitored at 250 nm using the PDA detector. Mass spectrometric analysis was performed under +ve ESI mode. The total ion chromatogram, peak area% and mass spectrum of the individual peak which was appeared in LC along with the full scan (*m/z* 50-800) were recorded. The total ion chromatogram and mass spectrum of the individual peak were recorded.

The natural abundance of C, O, Mg, and H isotope can be predicted from the comparison of the relative abundance of the isotope peak with respect to the base peak. The values of the natural isotopic abundance of the common elements are obtained from the literature [36-39]. The isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and Biofield Energy Treated magnesium gluconate was calculated.

Percentage (%) change in isotopic abundance ratio = $[(IAR_{Treated} - IAR_{Control})/ IAR_{Control}) \times 100]$

Where $IAR_{Treated}$ = isotopic abundance ratio in the treated sample and $IAR_{Control}$ = isotopic abundance ratio in the control sample.

Nuclear Magnetic Resonance (NMR) Analysis: 1H NMR spectra were recorded at 400 MHz on Agilent-MRDD2 FT-NMR. Approximately 3 mg of the sample was dissolved in DMSO-d6. Chemical shifts (d) were in parts per million (ppm) relative to the solvent's residual proton chemical shift {(CD3)2SO, δ = 2.5}. 1H NMR multiplicities were designated as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), multiplet (m), broad (br), apparent (app). 13C NMR spectra were measured at 100 MHz on Agilent-MRDD2 FT-NMR spectrometer at room temperature. Approximately 25 mg of the sample was dissolved in DMSO-d6. Chemical shifts (d) were in parts per million (ppm) relative to the solvent's residual carbon chemical shift {(CD3)2SO, δ = 39.52}.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

The LC-MS chromatograms of the control and The Trivedi Effect®-Biofield Energy Treated magnesium gluconate showed two sharp peaks (Figure 1) at the retention time (RT) of 1.53 and 1.67 minutes. The major %peak area of the Biofield Energy Treated sample (79.27%) was very close to the control sample (78.38%). This result concluded that the polarity of the Biofield Energy Treated sample remained same compared with the control sample.

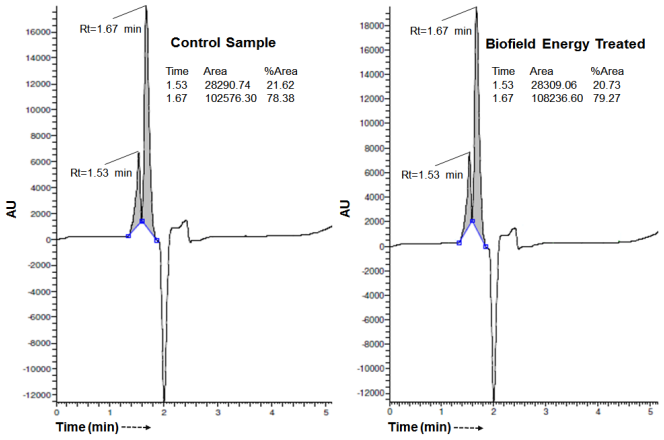

Figure 1: Total ion chromatograms (TIC) of the control and Biofield Energy Treated magnesium gluconate.

The magnesium gluconate samples were ionized *via* ESI in +ve ion mode. The ESI-MS spectra of the control and Biofield

Energy Treated samples of magnesium gluconate at Rt of 1.67 minutes (Figure 1) exhibited the presence of the molecular mass of magnesium gluconate adduct with hydrogen ion at *m/z* 415 (calcd for $C_{12}H_{23}MgO_{14}^{+}$, 415.09). Other higher mass peak at *m/z* 633.96 (calcd for $C_{18}H_{34}Mg_2O_{21}^{+}$, 634.13) in the control sample and at *m/z* 635.9 (calcd for $C_{18}H_{36}Mg_2O_{21}^{+}$, 636.14) in the Biofield Energy Treated Sample were observed (Figure 2). Many fragment ion peaks in the lower *m/z* region other than the protonated magnesium gluconate ion $[M+H]^{+}$ (*m/z* 415) were observed in both the control and Biofield Energy Treated samples (Figures 2 & 3) at *m/z* 397 (calcd for $C_{12}H_{21}MgO_{13}^{+}$, 397.08), 317 (calcd for $C_{10}H_{13}MgO_{10}^{+}$, 317.04), 219 (calcd for $C_6H_{11}MgO_7^{+}$, 219.03), and 179 (calcd for $C_6H_{11}O_6^{+}$, 179.06).

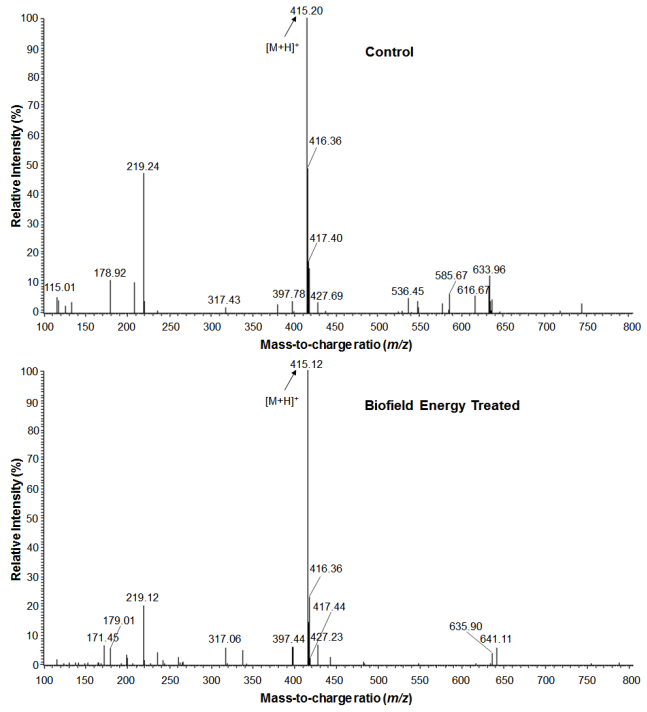

Figure 2: The ESI-MS spectra of the control and Biofield Energy Treated magnesium gluconate at R_t 1.67 minutes in the chromatograms.

The ESI-MS spectra of the control and Biofield Energy Treated magnesium gluconate (Figure 3) displayed almost similar type of mass fragmentation pattern. The molecular ion peak at *m/z* 415 exhibited 100% relative peak intensity in both ESI-MS spectra (Figure 2). The relative peak intensities of the other ion peaks in The Trivedi Effect® -Biofield Energy Treated sample were significantly altered compared to the control sample.

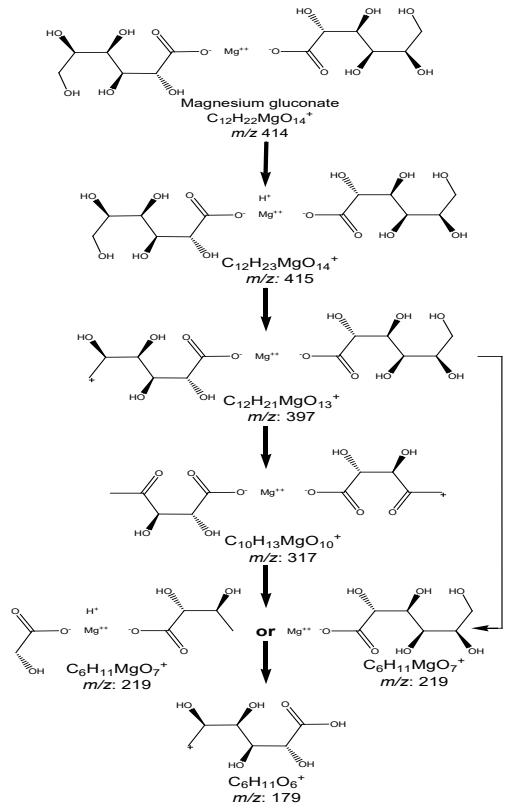

Figure 3: Proposed fragmentation pattern of control and Biofield Energy Treated magnesium gluconate.

Isotopic Abundance Ratio Analysis

The ESI-MS (+ve mode) spectra of the control and Biofield Energy Treated samples of magnesium gluconate showed the mass of a protonated molecular ion at *m/z* 415 ($C_{12}H_{23}MgO_{14}^{+}$) with 100% relative abundance. The theoretical calculation of isotopic peak P_{M+1} for the protonated magnesium gluconate presented as below:

P (^{13}C) = [(12 x 1.1%) x 100% (the actual size of the M+ peak)] / 100% = 13.20%

P (^{2}H) = [(23 x 0.015%) x 100%] / 100%= 0.35%

P (^{25}Mg) = [(1 x 12.66%) x 100%] / 100% = 12.66%

P (^{17}O) = [(14 x 0.04%) x 100%] / 100% = 0.56%

P_{M+1} i.e.^{13}C, ^{2}H, ^{17}O, and ^{25}Mg contributions from $(C_{12}H_{23}MgO_{14})^{+}$ to *m/z* 416 = 26.77%

Similarly, the theoretical calculation of isotopic peak P_{M+2} for the protonated magnesium gluconate presented as below:

P (^{26}Mg) = [(1 x 13.94%) x 100%] / 100% = 13.94%

P (^{18}O) = [(14 x 0.20%) x 100%] / 100% = 2.8%

P_{M+2} i. e.^{18}O, and ^{26}Mg contributions from $(C_{12}H_{23}MgO_{14})^{+}$ to *m/z* 416 = 16.74%

The calculated isotopic abundance of P_{M+1} value (26.77%) was lower to the observed value (36.57%), but the P_{M+2} value (16.74%) was close to the observed value (15%) (Table 1). Thus, the probability of A + 1 and A + 2 elements having an isotope with one and two mass unit heavier, respectively than the most abundant isotope (i.e. ^{13}C, ^{2}H, ^{17}O, ^{25}Mg, ^{18}O, and ^{26}Mg) contributions to the mass of the isotopic molecular ion $[M+1]^+$ and $[M+2]^+$. Deuterium did not contribute much any isotopic *m/z* ratios because of less natural abundance compared to the natural abundances of C, Mg, and O isotopes [38,40]. From the calculations, it is confirmed that ^{13}C, ^{18}O, ^{25}Mg, and ^{26}Mg have the major contributions from magnesium gluconate to the isotopic mass peak at *m/z* 416 and 417. Therefore, P_M, P_{M+1}, and P_{M+2} of the magnesium gluconate at *m/z* 415, 416, and 417 of the control and Biofield Energy Treated samples were obtained from the experimental relative abundance of $[M^+]$, $[(M+1)^+]$, and $[M+2]^+$ peaks, respectively in the ESI-MS spectra (Table 1).

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 415.2 (%)	100	100
P_{M+1} at *m/z* 416.4 (%)	36.57	14.48
P_{M+1}/P_M	0.3657	0.1448
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		-60.405
P_{M+2} at *m/z* 417.4 (%)	15	3.1
P_{M+2}/P_M	0.15	0.031
% Change of isotopic abundance ratio (P_{M+2}/P_M) with respect to the control sample		-79.333

P_M = the relative peak intensity of the parent molecular ion M^+; P_{M+1} = the relative peak intensity of the isotopic molecular ion $[M+1]^+$, P_{M+2} = the relative peak intensity of the isotopic molecular ion $[M+2]^+$, and M = mass of the parent molecule.

Table 1: LC-ESI-MS isotopic abundance ratio analysis of control and Biofield Energy Treated magnesium gluconate.

The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated magnesium gluconate was significantly decreased by 60.41% compared to the control sample (Table 1). Thus, the ^{13}C, ^{2}H, ^{17}O, and ^{25}Mg contributions from $(C_{12}H_{23}MgO_{14})^+$ to *m/z* 416 in the Biofield Energy Treated sample was significantly decreased compared to the control sample. Similarly, the isotopic abundance ratio of P_{M+2}/P_M in the Biofield Energy Treated sample was significantly decreased by 79.33% compared to the control sample (Table 1). Therefore, the ^{18}O, and ^{26}Mg contributions from $(C_{12}H_{23}MgO_{14})^+$ to *m/z* 417 in the Biofield Energy Treated magnesium gluconate significantly decreased compared with the control sample.

Increased or decreased isotopic abundance of the compounds indicated the change in the number of neutrons in the molecule. So, it can be assumed that due to the possible mediation of neutrinos oscillation may alter the atomic mass and the atomic charge. Thus, it is expected that The Trivedi Effect®-Consciousness Energy Healing Treatment might provide the necessary energy for the neutrino oscillations leads to the modification of the fundamental physicochemical properties of a compound [31-33]. The altered kinetic isotope effects change the isotopic abundance ratio of the atoms in the reactants in a chemical reaction, which is very useful to study the reaction mechanism, understand the enzymatic transition state, and all aspects of enzyme mechanism that is supportive for designing effective and specific inhibitors [31-33,35]. In animal body magnesium is an essential cofactor for various enzymatic reactions, The Trivedi Effect® Treated magnesium gluconate that had the decreased isotopic abundance ratio (P_{M+1}/P_M and P_{M+2}/P_M) might be advantageous for the faster chemical reactions, the study of enzyme mechanism, and in the design of novel potent enzyme inhibitors.

Nuclear Magnetic Resonance (NMR) Analysis

Figures 5 and 6 shown the spectra of ^{1}H and ^{13}C NMR, respectively for the control and Biofield Energy Treated magnesium gluconate. The ^{1}H and ^{13}C NMR data of both the control and Biofield Energy Treated magnesium gluconate are presented in Table 2. The ^{1}H NMR spectra the control and Biofield Energy Treated samples (Figure 4) indicated that signals for the protons coupling of CH_2, CH, and OH protons of magnesium gluconate were in the range of δ 3.4 to 6.4 ppm (Table 2), which was similar to the reported literature [41,42]. Similarly, the carbon signals for CH_2, CH, and CO groups in the ^{13}C NMR spectrum (Figure 5) of the Biofield Energy Treated sample were almost same compared to the control sample (Table 2). The NMR results indicated that there was no structural modification of the Biofield Energy Treated magnesium gluconate compared to the control sample.

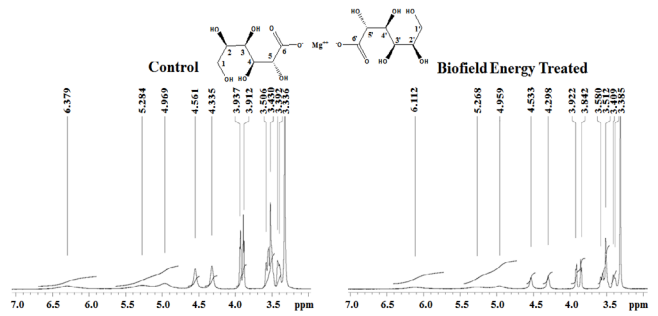

Figure 4: The ^{1}H NMR spectra of the control and Biofield Energy Treated magnesium gluconate.

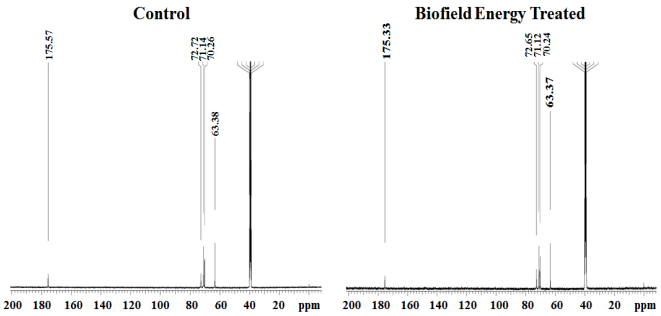

Figure 5: The ^{13}C NMR spectra of the control and Biofield Energy Treated magnesium gluconate.

Position	^{1}H NMR d (ppm)			^{13}C NMR d (ppm)	
	Number	Control	Treated	Control	Treated
1, 1'	4H (CH_2) & 2H(OH)	3.92 (d, *J* = 10 Hz) & 6.38 (br, s)	3.88 (d, *J* = 10 Hz) & 6.11 (br, s)	63.38	63.37
2, 2'	2H (CH) & 2H(OH)	3.57 (br, t, *J* = 30.4 Hz) & 4.56 (s)	3.55 (br, t, *J* = 27.2 Hz) & 4.53 (s)	70.26	70.24
3, 3'	2H (CH) & 2H(OH)	3.57 (br, t, *J* = 30.4 Hz) & 5.13 (br, d)	3.55 (br, t, *J* = 27.2 Hz) & 5.11 (br, d)	NA	NA
4, 4'	2H (CH) & 2H(OH)	3.57 (br, t, *J* = 30.4 Hz) & 5.13 (br, d)	3.55 (br, t, *J* = 27.2 Hz) & 5.11 (br, d)	71.14	71.12
5, 5'	2H (CH) & 2H(OH)	3.36 (br, t, *J* = 22.4 Hz) & 4.34 (br, s)	3.40 (br, t, *J* = 9.6 Hz) & 4.30 (br, s)	72.72	72.65
6, 6'	--	--	--	175.28	175.33

br- broad, s- singlet, t- triplet, d-doublet and NA: not available.

Table 2: ^{1}H and ^{13}C NMR spectroscopic data of both the control and Biofield Energy Treated magnesium gluconate.

Conclusion

The study evaluated the influence of The Trivedi Effect® - Consciousness Energy Healing Treatment on magnesium gluconate sample using sophisticated analytical techniques. The LC-ESI-MS analysis of the control and Biofield Energy Treated samples exhibited the presence of the molecular mass of magnesium gluconate adduct with hydrogen ion at *m/z* 415 (calcd for $C_{12}H_{23}MgO_{14}^{+}$, 415.09) at the retention time of 1.67 minutes and almost similar fragmentation pattern. But, the relative peak intensities of the fragmented ions of the Biofield Energy Treated sample were significantly changed compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{25}Mg/^{24}Mg$) in the Biofield Energy Treated magnesium gluconate was significantly decreased by 60.41% compared to the control sample. Therefore, the ^{13}C, ^{2}H, ^{17}O, and ^{25}Mg contributions from $(C_{12}H_{23}MgO_{14})^{+}$ to *m/z* 416 in the Biofield Energy Treated sample was significantly decreased compared to the control sample. Similarly, the isotopic abundance ratio of P_{M+2}/P_M ($^{18}O/^{16}O$ or $^{26}Mg/^{24}Mg$) in the Biofield Energy Treated sample was significantly decreased by 79.33% compared to the control sample. Briefly, the ^{18}O and ^{26}Mg contributions from $(C_{12}H_{23}MgO_{14})^{+}$ to *m/z* 417 in the Biofield Energy Treated magnesium gluconate significantly decreased compared with the control sample. The Trivedi Effect®-Biofield Energy Healing Treated magnesium gluconate might exhibit isotope effects such as altered physicochemical and thermal properties, the rate of reaction, selectivity and binding energy due to its reduced isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M compared with the control sample. Thus, The Trivedi Effect®-Consciousness Energy Healing Treated magnesium gluconate might be supportive to design the novel potent enzyme inhibitors and better pharmaceutical and nutraceutical formulations by using its kinetic isotope effects. The Trivedi Effect®-Energy of Consciousness Healing Treatment could be a useful and economical approach in the design of better nutraceutical and pharmaceutical formulations which can offer significant therapeutic responses against septic shock, arrhythmias, diabetes mellitus, cancer, asthma, allergies, inflammatory diseases, immunological disorders, gestational hypertension, etc.

Acknowledgements

The authors are grateful to GVK Biosciences Pvt. Ltd., Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for their assistance and support during this work.

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