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Research Article

The Isotopic Abundance Ratio Analysis of the Consciousness Energy Healing Treated Berberine Chloride Using LC-MS and GC-MS Analytical Techniques

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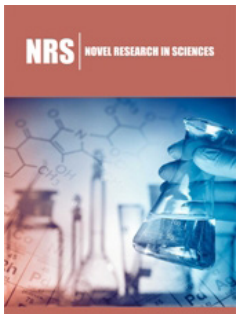

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Abstract

Berberine is a benzylisoquinoline alkaloid which has enormous therapeutic potential, but the bioavailability is very poor due to its low solubility and poor intestinal absorption. In this study, the impact of the Trivedi Effect®-Biofield Energy Healing Treatment on the structural properties and the isotopic abundance ratio of berberine chloride was evaluated using advanced spectroscopic methods. Berberine chloride sample was divided into control and treated parts. Only the treated part was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a famous Biofield Energy Healer, Dahryn Trivedi. The LC-MS spectra of both the berberine chloride samples observed at retention time 2 minutes and the molecular ion peak at *m/z* 336.25 [M]+ (calculated for C20H18NO4+, 336.12). The LC-MS based isotopic abundance ratio of PM+1/PM in the treated berberine chloride was significantly decreased by 18.53% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of PM+1/PM in the treated berberine chloride was decreased by 4.69% compared with the control sample. Thus, 13C, 2H, 15N, and 17O contributions from (C20H18NO4)+ to *m/z* 337 in the treated sample were significantly decreased compared with the control sample. But, the isotopic abundance ratio of PM+2/PM in the treated sample was significantly increased by 11.52% compared with the control sample. Hence, 18O contribution from (C20H16NO4)3+ to *m/z* 336 in the treated sample was significantly increased compared with the control sample. The isotopic abundance ratios of PM+1/PM (2H/1H or 13C/12C or 15N/14N or 17O/16O) and PM+2/PM (18O/16O) in the treated berberine chloride were significantly altered compared to the control sample. The changes in isotopic abundance might be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The decreased isotopic abundance ratio (PM+1/PM) of the Consciousness Energy Healing Treated berberine chloride may decrease the chemical bond strength, influence its stability, and bioavailability in the body. The new form of berberine chloride would be more efficacious novel pharmaceutical formulations that might offer better solubility, dissolution, absorption, bioavailability and therapeutic response against diarrhoea, gastroenteritis, bacterial and fungal infections, cancer, arrhythmia, diabetes, hyperlipidemia, inflammation, etc.

Keywords: Consciousness energy healing treatment; Berberine chloride; The Trivedi Effect®; Biofield energy; LC-MS, GC-MS

Introduction

Berberine is an alkaloid found in many medicinal plants such as *Mahonia aquifolium*, *Coptis chinensis*, *Berberis vulgaris*, *Hydrastis Canadensis*, etc. [1,2]. Traditionally it has been used as a natural dye. Many research suggested that berberine treat diarrhoea, gastroenteritis, bacterial, fungal and other microbial infections, cancer, arrhythmia, diabetes, hyperlipidemia, inflammation, etc. [3-13]. It has anti-aging and anti-glycaemic properties [14]. Due to the multiple biological activities with less toxicity and low cost, berberine has recently gained great interest for the treatment of human diseases [15-18]. Although berberine has great therapeutic potential as a drug molecule, its bioavailability is very poor (less than 1%), which make a challenge to develop it as a clinical candidate. The poor bioavailability of the berberine is due to its low solubility, poor membrane permeability, poor intestinal absorption, and rapid biotransformation also account for the low plasma concentrations [19,20].

Many underway researches are going on for the improvement of bioavailability of berberine [21]. The Biofield Energy Healing Treatment (the Trivedi Effect®) has a considerable impact on the particle size, surface area, thermal behaviour, along with bioavailability of the pharmaceutical/nutraceutical compounds [22-25]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a person can harness this energy from the Universe and transfer it anywhere on the planet via the possible mediation of neutrinos [26]. The Biofield is an electromagnetic field around the human body generated by continues moment of the charged particle in the body (particles, ions, cells, blood/lymph flow, brain functions, and heart function) [25,27]. The use of energy medicine has been studied and reported with the significant outcome in different disease conditions [28,29]. The process of the energy harness from the universe and transfer into any living and non-living object(s) is called the Biofield Energy Healing Treatment [30,31]. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Energy Therapy as a Complementary and Alternative Medicine (CAM) health care approach along with the other therapies, medicines, and practices such as Qi Gong, Tai Chi, homeopathy, yoga, hypnotherapy, Reiki, etc. [32]. These CAM therapies have been accepted by most of the U.S.A. people [33]. The Trivedi Effect® also reported with significant impact on the physicochemical/biological properties of the several living and non-living object(s), i.e., organic compounds, metals, ceramic, crops, microbes [34-38], etc.

The analysis of stable isotope ratio has various applications for the understanding of isotope effects resulting from the variation of the isotopic composition of the molecule [39,40]. Isotope ratio analysis can be performed by using the conventional mass spectrometry techniques, i.e., gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [40,41]. The Consciousness Energy Healing Treatment has also found altering the isotopic abundance ratio of the chemical compounds [42,43]. Thus, in this study the LC-MS and GC-MS were used to characterize the structural properties and evaluated the isotopic abundance ratio analysis of PM+1/PM and PM+2/PM in the Consciousness Energy Healing Treated berberine chloride as compared to the control sample.

Materials and Methods

Chemicals and reagents

The test sample berberine chloride hydrate (98.1% HPLC) was purchased from Tokyo Chemical Industry Co. Ltd., Japan and other reagents used in the experiment were purchased in India.

Consciousness energy healing treatment strategies

The test sample berberine chloride was divided into two parts. One part of the berberine chloride was received the Trivedi Effect®-Consciousness Energy Healing Treatment under standard laboratory conditions known as the Biofield Energy Treated berberine. The treatment was provided remotely for 3 minutes through the healer's unique energy transmission process by the renowned Biofield Energy Healer, Dahryn Trivedi, USA, to the test sample berberine chloride. The other part of the berberine did not receive the Biofield Energy Treatment but was treated with a "sham" healer who did not have any knowledge about the Biofield Energy and its treatment procedure. After all, both the samples were kept in sealed conditions and characterized with the help of LC-MS and GC-MS analytical techniques.

Characterization

Liquid chromatography-mass spectrometry (LC-MS) analysis and calculation of isotopic abundance ratio

The LC-MS analysis of both the berberine chloride sample was carried out with the help of LC-MS Thermo Fisher Scientific, the USA, connected with an ion trap detector and triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6mm X 5 micron) and column temperature maintained at 35 °C. Acetonitrile and methanol were used as diluent for the sample preparation. Berberine chloride solution (5µL) was injected and the analyte was eluted using acetonitrile+0.1% formic acid (50:50) pumped at a constant flow rate of 1mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 10min, where peaks were monitored at 210nm using the PDA detector. The mass spectrometric analysis was performed in +ve ESI mode.

The natural abundance of each isotope (C, H, N, and O) can be predicted from the comparison of the height 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 [40, 44-46]. The LC-MS based isotopic abundance ratios (PM+1/PM) for the control and treated berberine chloride was calculated using equation (1).

(%) Change in isotopic abundance ratio = [(IARTreated – IARControl)/ IARControl)]x 100 (1)

Where IARTreated = isotopic abundance ratio in the treated sample and IARControl = isotopic abundance ratio in the control sample.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS of both the samples of berberine chloride was analyzed with the help of Perkin Elmer GC equipped with a PE-5MS (30M x 250 micros x 0.250 microns) capillary column and coupled to a single quadrupole mass detector was operated with electron impact (EI) ionization in +ve mode. The oven temperature was from 75 °C (5 min hold) to 280 °C (14.5 min hold) @ 10 °C /min (total run time 40 min). The sample was prepared taking 50 mg of the berberine chloride in 2.5ml methanol as a diluent. The GC-MS based isotopic abundance ratios (PM+1/PM and PM+2/PM) for the control and treated berberine chloride was calculated using equation (1).

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS)

The chromatograms of both the samples of berberine chloride showed a single major chromatographic peak at retention time (R_t) of ~2.0 minutes (Figure 1). This indicated that the polarity of both the samples may be similar. Similarly, the mass spectra of berberine chloride exhibited the mass of the molecular ion peak at *m/z* 336.3 [M]+ (calculated for C20H18NO4+, 336.12) along with other fragmentation peaks at 321.42 (C19H15NO42+) and *m/z* 192.1 (C14H10N3+) in both the control and treated samples (Figures 2 & Figure 3). The mass spectra of berberine chloride show the molecular peak [M]+ at *m/z* 336 in the mass spectrum in +ve ion mode [47].

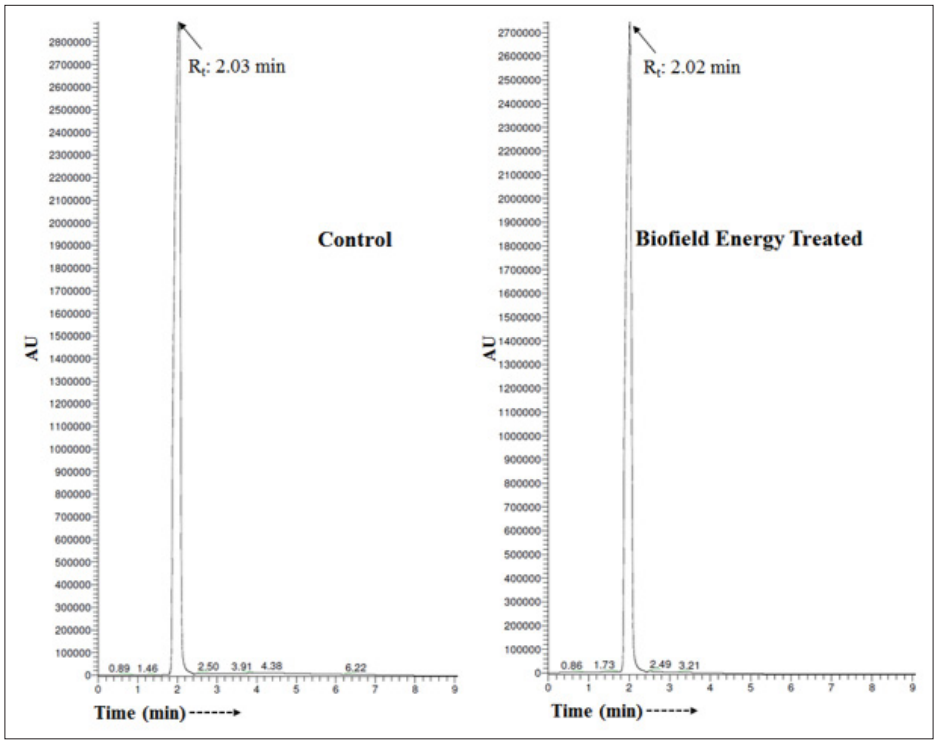

Figure 1: Liquid chromatograms of the control and biofield energy treated berberine chloride.

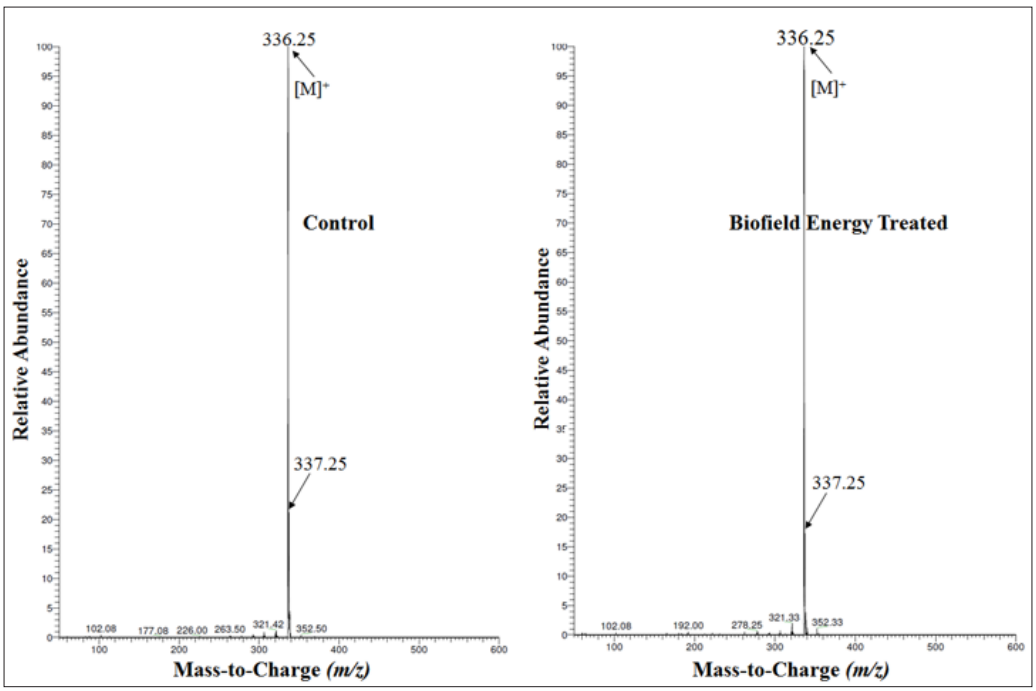

Figure 2: Mass spectra of the control and treated berberine chloride at R_t ~2.0 minutes.

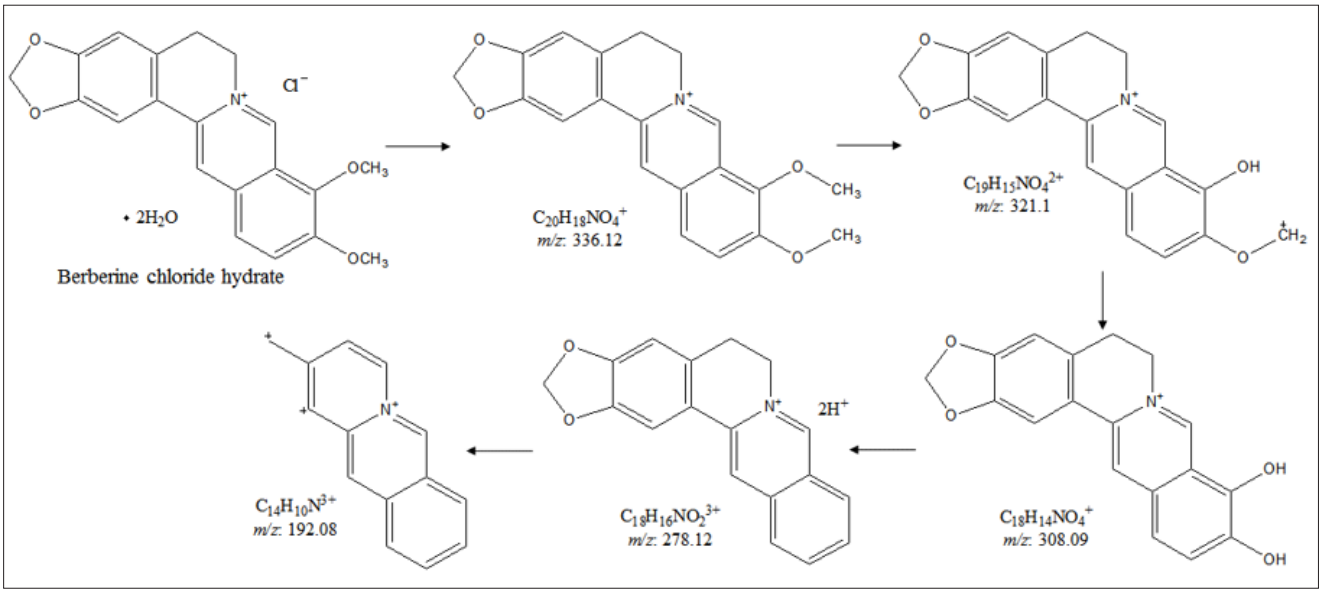

Figure 3: Proposed fragmentation pattern of berberine chloride.

The LC-MS spectra of both the samples showed the mass of the molecular ion peak at *m/z* 336.25 [M]+ (calculated for C20H18NO4+, 336.12) with 100% relative intensity. The theoretical calculation of PM+1 for berberine chloride was presented as below:

P (13C) = [(20 x 1.1%) x 100% (the actual size of the M- peak)] / 100% = 22%

P (2H) = [(18 x 0.015%) x 100%] / 100%= 0.27%

P (15N) = [(1 x 0.4%) x 100%] / 100% = 0.4%

P (17O) = [(4 x 0.04%) x 100%] / 100% = 0.16%

PM+1, i.e. 13C, 2H, 15N, and 17O contributions from (C20H18NO4)+ to *m/z* 337 = 22.83%

From the above calculation, it has been found that 13C, and 15N have the major contribution to *m/z* 337.

Based on the LC-MS data, the isotopic abundance ratio analysis PM and PM+1 near *m/z* 336 [M+] and 337 [(M+1)+] of the control and treated berberine chloride samples were evaluated (Table 1). The isotopic abundance ratio (PM+1/PM) in the treated berberine chloride was significantly decreased by 18.53% compared with the control sample (Table 1). Thus, it was concluded that the 13C, 2H, 15N, and 17O contributions from (C20H18NO4)+ to *m/z* 337 in the Biofield Energy Treated sample might have significantly increased compared to the control sample.

Table 1: LC-MS based isotopic abundance analysis results in biofield energy treated berberine chloride compared to the control sample.

Parameter	Control Sample	Biofield Energy Treated Sample
P_M at *m/z* 336 (%)	100	100
P_{M+1} at *m/z* 337 (%)	21.16	17.24
P_{M+1}/PM	0.21	0.17
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		-18.53

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)+]; M: mass of the parent molecule.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The berberine chloride samples showed the presence of a sharp chromatographic peak at R_t 21.5 min in the GC-MS chromatograms (Figures 4 & Figure 5). The parent molecular ion peak of berberine chloride at *m/z* 334 [M]+ (calculated for C20H16NO43+, 334.11) was observed in both the samples, along with the lower mass fragment ion peaks (Figures 4 & Figure 5).

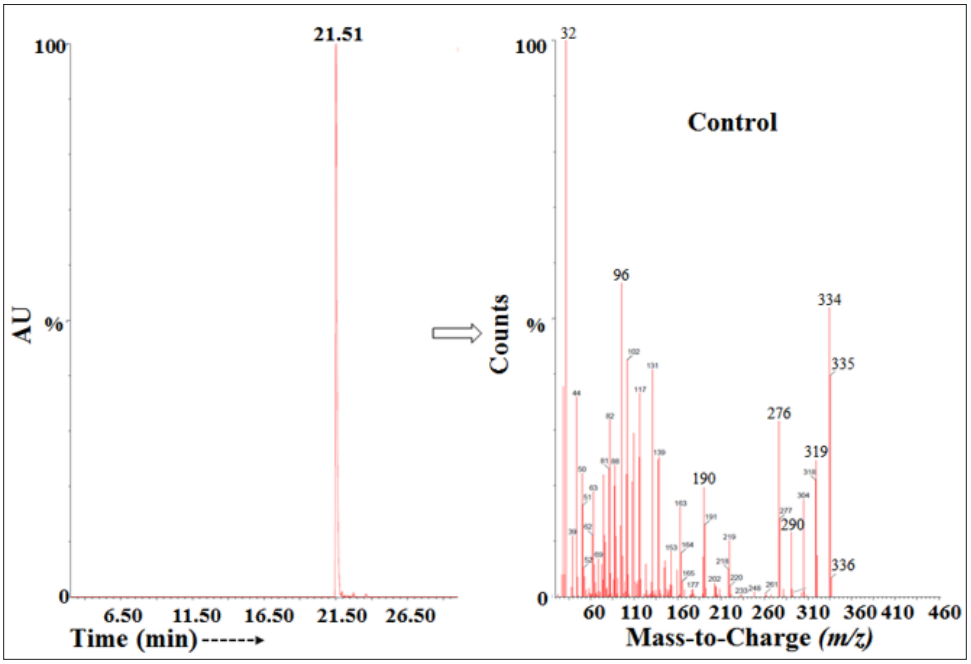

Figure 4: The GC-MS chromatogram and mass spectra of the control berberine chloride.

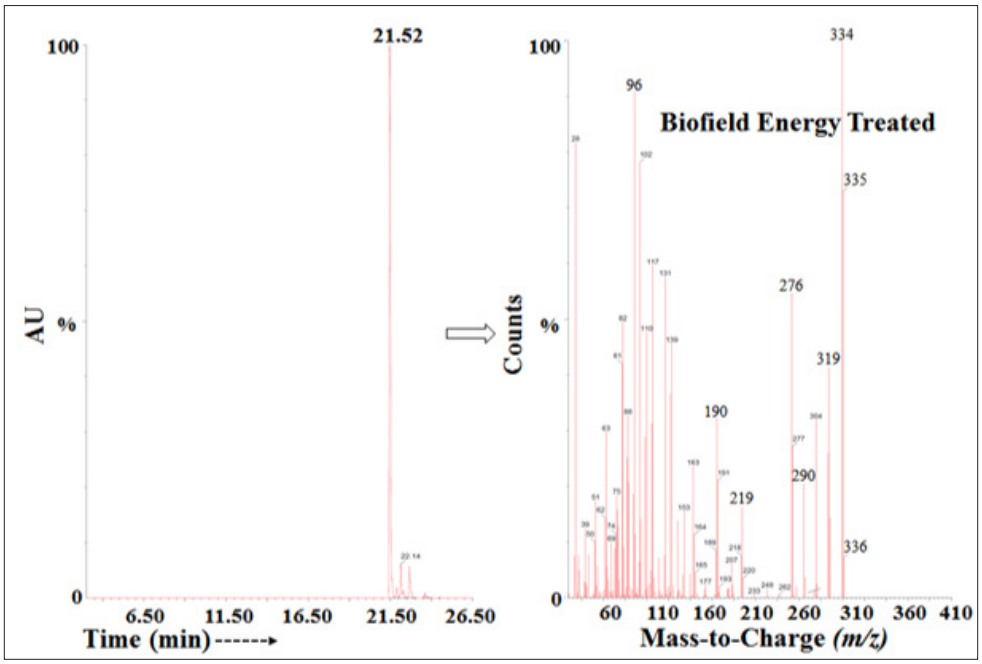

Figure 5: The GC-MS chromatogram and mass spectra of the biofield energy treated berberine chloride.

The mass spectra of both the samples showed the molecular ion peak [M]+ at *m/z* 334 [M]+ (calculated for C20H16NO43+, 334.11). The theoretical calculation of PM+1 and PM+2 for berberine chloride was presented as below:

P (13C) = [(20 x 1.1%) x 51.96% (the actual size of the M+ peak)] / 100% = 11.43%

P (2H) = [(16 x 0.015%) x 51.96%] / 100%= 0.12%

P (15N) = [(1 x 0.4%) x 51.96%] / 100% = 0.21%

P (17O) = [(4 x 0.04%) x 51.96%] / 100% = 0.08%

PM+1, i.e. 13C, 2H, 15N, and 17O contributions from (C20H16NO4)3+ to *m/z* 335 = 11.84%

Similarly,

P (18O) = [(4 x 0.2%) x 51.96%] / 100% = 0.42%

PM+2, i.e. 18O contributions from (C20H16NO4)3+ to *m/z* 336 = 0.12%

From the above calculation, it has been found that 13C, 15N, and 18O have major contribution to *m/z* 335 and 336.

The GC-MS based isotopic abundance ratios analysis of the treated berberine chloride were calculated compared to the control sample. PM, PM+1, and PM+2 for berberine chloride near *m/z* 334 [M+], 335 [(M+1)+], and 336 [(M+2)+], respectively of both the samples were calculated (Table 2). The isotopic abundance ratio of PM+1/PM in the treated berberine chloride was decreased by 4.69% compared with the control sample (Table 2). Hence, 13C, 2H, 15N, and 17O contributions from (C20H16NO4)3+ to *m/z* 335 in the treated sample were significantly increased compared with the control sample. Whereas, the isotopic abundance ratio of PM+2/PM in the treated berberine chloride was significantly increased by 11.52% compared with the control sample (Table 2). Hence, 18O contribution from (C20H16NO4)3+ to *m/z* 336 in the treated berberine chloride were significantly increased compared with the control sample.

Table 2: GC-MS based isotopic abundance analysis results of biofield energy treated berberine chloride compared to the control samples.

Parameter	Control Sample	Biofield Energy Treated Sample
P_M at *m/z* 334 (%)	51.96	100
P_{M+1} at *m/z* 335 (%)	39.76	72.93
P_{M+1}/P_M	0.77	0.73
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		-4.69
P_{M+2} at *m/z* 336 (%)	3.35	7.19
P_{M+2}/P_M	0.06	0.07
% Change of isotopic abundance ratio (P_{M+2}/P_M) with respect to the control sample		11.52

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)+]; M: mass of the parent molecule.

The chromatographic and spectroscopic study confirmed the structure of berberine. The isotopic abundance ratios of PM+1/PM (2H/1H or 13C/12C or 15N/14N or 17O/16O) and PM+2/PM (18O/16O) in the treated berberine chloride were significantly altered compared to the control sample. The altered isotopic composition in the molecular level of the treated berberine chloride might be due to the altered neutron to proton ratio in the nucleus. The changes in isotopic abundance might be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. A neutrino is an elementary particle which interacts only via the weak subatomic force and gravity [48]. The neutrinos change identities and it is only possible if the neutrinos possess mass and this particle has the ability to interact with protons and neutrons in the nucleus, which specified a close relation between neutrino and the isotope formation [26,40,41]. The isotopic abundance ratios 2H/1H, 13C/12C, 15N/14N or 17O/16O or 18O/16O would highly influence the atomic bond vibration of treated berberine chloride [49]. The decreased isotopic abundance ratio (PM+1/PM) of the Consciousness Energy Healing Treated berberine chloride may decrease the chemical bond strength, influence its stability, and bioavailability in the body. The overall results concluded that the Trivedi Effect®-Consciousness Energy Healing Treatment might create a new form of berberine chloride which would be better for the prevention and treatment of various diseases, i.e., diarrhea, gastroenteritis, bacterial, fungal and other microbial infections. It would also help for the treatment of cancer, arrhythmia, diabetes, hyperlipidemia, and inflammation.

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratios (PM+1/PM, PM+2/PM) of berberine chloride. The LC-MS spectra of both the control and treated berberine chloride samples observed at retention time 2.0 minutes and the molecular ion peak at *m/z* 336.25 [M]+ (calculated for C20H18NO4+, 336.12). The LC-MS based isotopic abundance ratio of PM+1/PM in the Consciousness Energy Healing Treated berberine chloride was significantly decreased by 18.53% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of PM+1/PM in the Consciousness Energy Healing Treated berberine chloride was decreased by 4.69% compared with the control sample. Thus, 13C, 2H, 15N, and 17O contributions from (C20H18NO4)+ to *m/z* 337 in the Consciousness Energy Healing Treated sample were significantly decreased compared with the control sample. But, the isotopic abundance ratio of PM+2/PM in the Consciousness Energy Healing Treated sample was significantly increased by 11.52% compared with the control sample. Hence, 18O contribution from (C20H16NO4)3+ to *m/z* 336 in the Consciousness Energy Healing Treated sample were significantly increased compared with the control sample.

The isotopic abundance ratios of PM+1/PM (2H/1H or 13C/12C or 15N/14N or 17O/16O) and PM+2/PM (18O/16O) in the Consciousness Energy Healing Treated berberine chloride were significantly altered compared to the control sample. The changes in isotopic abundance might be due to changes in nuclei possibly

through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The decreased isotopic abundance ratio (PM+1/PM) of the Consciousness Energy Healing Treated berberine chloride may decrease the chemical bond strength, influence its stability, and bioavailability in the body. The new form of the Biofield Energy Treated berberine chloride would be more efficacious novel pharmaceutical formulations that might offer better solubility, dissolution, absorption, bioavailability and therapeutic response against diarrhoea, gastroenteritis, bacterial and fungal infections, cancer, arrhythmia, diabetes, hyperlipidemia, inflammation, etc.

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