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Evaluation of the Isotopic Abundance Ratio of Consciousness Energy Healing Treated L-Tryptophan Using LC-MS Spectrometry

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Abstract

L-tryptophan is an essential α-amino acid, necessary for the normal growth in newborns, nitrogen balance in adults, protein synthesis, precursor of serotonin, melatonin, niacin, and albeit inefficiently in human, also the precursor of indole alkaloids and auxins in plants. This current study was designed to investigate the impact of the Trivedi Effect®-Biofield Energy Healing Treatment (Blessing) on the structural properties and the isotopic abundance ratio of L-tryptophan using LC-MS analytical technique. L-tryptophan sample was divided into two parts, one part of L-tryptophan was considered as the control sample (no Biofield Energy Treatment was provided), while the second part was treated with the Trivedi Effect®-Consciousness Energy Healing Treatment/ Blessing remotely by a renowned Biofield Energy Healer, Dahryn Trivedi and termed as the treated sample. The mass spectra of both the control and treated samples with respect to the chromatographic peak at retention time (R_t) 2.1 minutes exhibited the mass of the molecular ion peak adduct with hydrogen ion at *m/z* 205.08 (calcd for $C_{11}H_{13}N_2O_2^+$, 205.1), along with low molecular fragmented mass peaks at *m/z* 188, 159, and 102 for $C_{11}H_{12}N_2O^{2+}$, $C_{10}H_{11}N_2^+$, and $C_8H_6^+$, respectively were also observed. The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in the treated L-tryptophan was significantly increased by 35.93% compared with the control sample. Hence, the ^{13}C, ^2H, ^{15}N, and ^{17}O contributions from $C_{11}H_{13}N_2O_2^+$ to *m/z* 206.08 in the treated L-tryptophan was significantly increased compared to the control sample. It could be hypothesized that the changes in the isotopic abundance and mass peak intensities due to the modification in nuclei possibly through the interference of neutrino particles using the Trivedi Effect®-Consciousness Energy Healing Treatment. The Biofield Energy Treated/Blessed L-tryptophan with increased stable isotopic abundance ratio might have changed the physicochemical properties with higher force constant in the molecule. The new form of treated L-tryptophan would be a better and more stable in the supplements, nutraceutical, and pharmaceutical formulations, which would be advantageous for the prevention and treatment of pellagra, depression, kynurenine. It could also maintain the normal label of tryptophan and avoid increase of its metabolite, lower the neurotoxin and a metabotoxin behavior, glutaric aciduria type I (glutaric acidemia type I) disorder, eosinophilia-myalgia syndrome (EMS), incurable and sometimes fatal flu-like neurological condition, etc. As tryptophan is the precursor for the plant hormones like indole alkaloids and auxins, hence, this treated L-tryptophan would be advantageous for the improvement of yield, productivity, and quality of crops and other plants.

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Introduction

L-tryptophan [$C_{11}H_{12}N_2O_2$] is an essential α-amino acid, which must be obtained from the diet and supplements. It is necessary for normal growth in new-borns, nitrogen balance in adults, protein synthesis, a precursor of serotonin, melatonin, niacin, and albeit inefficiently in human. It is also the precursor of indole alkaloids and auxins in plants [1, 2]. It is converted to 5-hydroxy-tryptophan (5-HTP), which increase the production of serotonin, a neurotransmitter essential in regulating sleep, appetite, mood, temperature, sexual behaviour, and pain [1, 3]. Sources for tryptophan are red meat, eggs, fish, poultry, brown rice, soybeans, chocolate, oats, dried dates, milk, yogurt, cottage cheese, sesame, chickpeas, almonds, sunflower seeds, pumpkin seeds, buckwheat, spirulina, peanuts, etc. [1, 2]. The daily requirement of tryptophan for adults is 3 mg/kg/day. The tryptophan and protein requirement decreases with age. Improper diet, high maize or other tryptophan-deficient diets, fructose malabsorption, Hartnup's disease, *etc.* are the cause for reduced levels of tryptophan in the blood. Tryptophan deficient can be the cause of pellagra; the other deficiency diseases are depression and kynurenine. Under certain situations if the label of tryptophan and its metabolite increase, it can behave like a neurotoxin and a metabotoxin, glutaric aciduria type I (glutaric acidemia type I) disorder, eosinophilia-myalgia syndrome (EMS), create an incurable and sometimes fatal flu-like neurological condition, etc. [1, 2, 5-8]. L-tryptophan is slightly soluble in water, very slightly soluble in alcohol, practically insoluble in ether and chloroform. On heat to decompose it emits toxic fumes of nitric oxide [1].

The physicochemical properties of L-tryptophan are very important for the supplements, nutraceutical/ pharmaceutical, and other industries. The quality and efficiency of a pharmaceutical/ nutraceutical formulation depend upon the physicochemical properties of the substance, which is a challenging task for the scientific communities [9]. In this scenario, The Trivedi Effect®- Consciousness Energy Healing Treatment have the astonishing capabilities to transform the properties of many living and non-living object(s) [10-14]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a person can harness this inherently intelligent energy from the Universe and transmit it anywhere on the planet through the possible mediation of neutrinos [15]. Every living organism possesses a unique infinite, para-dimensional electromagnetic energy field surrounding the body known as Biofield Energy. The Biofield Energy Healers can harness the energy from the "Universal Energy Field" and can transmit into any living or non-living object(s), which is known as the Biofield Energy Healing Treatment. There are several Biofield based Energy Healing Therapies that are used nowadays against various disease conditions [16-18]. Biofield Energy Healing therapy has been recognized worldwide as a Complementary and Alternative Medicine (CAM) health care approach by National Center of Complementary and Integrative Health (NCCIH) with other therapies, medicines and practices such as Ayurvedic medicine, traditional Chinese herbs and medicines, homeopathy, yoga, chiropractic/osteopathic manipulation, Qi Gong, Tai Chi, meditation, acupressure, acupuncture, healing touch, hypnotherapy, naturopathy, Reiki, cranial sacral therapy, etc. [19]. These are the therapies have been well accepted by most of the U.S.A. population with several advantages [20]. The Trivedi Effect®-Consciousness Energy Healing/Blessing Treatment (Biofield Energy Healing Treatment) also reported with significant results altering the physicochemical properties of chemicals, metals, ceramics and polymers

[21-24], transformed antimicrobial properties [25, 26], improved agricultural crop yield, productivity, and quality [10, 11, 27], improved skin health [28, 29], improved the cancer in cancer cell line [30], improved bioavailability of pharmaceutical and nutraceutical compounds [31, 32], and altered the isotopic abundance ratio [33, 34].

Study of the natural stable isotope ratio analysis has many applications in the different field to understand the isotope effects resulting from the alterations of the isotopic composition [35-37]. Gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS), are the widely used sophisticated analytical techniques for the analysis of isotope ratio with sufficient precision [36]. The Trivedi Effect®-Consciousness Energy Healing Treatment/Blessing could be an economical approach to obtain a better desirable L-tryptophan with improved physicochemical properties for the supplements, nutraceutical, and pharmaceutical formulations. Therefore, this study was designed and evaluated the impact of the Trivedi Effect® - Consciousness Energy Healing Treatment/Blessing on L-tryptophan using LC-MS for the structural characterization and the isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) compared to the control sample.

Materials and Methods

Chemicals and Reagents

L-tryptophan (> 99%) was purchased from Alfa Aesar, India. All other chemicals used during the experiments were of analytical grade available in India.

Consciousness Energy Healing Treatment Strategies

The L-tryptophan powder sample was the test sample divided into two parts. One part of the test sample was considered as a control sample (no Biofield Energy Treatment was provided). However, the other part of the test sample was exposed to the Trivedi Effect®-Consciousness Energy Healing Treatment remotely under standard laboratory conditions for ~3 minutes and known as the Trivedi Effect® Treated/ Blessed (Biofield Energy Treated) L-tryptophan. The Biofield Energy Treatment/Blessing was provided through the healer's unique energy transmission process by the renowned Biofield Energy Healer, Dahryn Trivedi, USA, to the test sample. Further, the control sample was treated with "sham" healer for the better comparison. However, the "sham" healer did not have any knowledge about the Biofield Energy Treatment/Blessing. After that, the Biofield Energy Treated/Blessed and untreated L-tryptophan samples were kept in sealed conditions and characterized using LC-MS analytical techniques.

Characterization

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis and Calculation of Isotopic Abundance Ratio

The liquid chromatography-mass spectrometric analysis of the control and Biofield Energy Treated/ Blessed L-tryptophan was carried out with the help of LC-MS ThermoFisher Scientific, the USA equipped with an ion trap detector connected with a triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron), maintained at 25°C. The diluent used for the sample preparation was methanol. The L-tryptophan solution injection volume was 10 µL and the analyte was eluted using acetonitrile (80%) + 0.1% formic acid (20%) pumped at a constant flow rate of 1 mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 10 min. Peaks were monitored at 278 nm using the PDA detector. Mass spectrometric analysis was performed under ESI +ve ion 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* 50-400) were recorded. The total ion chromatogram and mass spectrum of the individual peak (appeared in LC-MS) were recorded.

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 [38-40]. The LC-MS based isotopic abundance ratio (P_{M+1}/P_M) for the control and Biofield Energy Treated L-tryptophan ($C_{11}H_{13}N_2O_2^+$) 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.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS)

The control and Biofield Energy Treated/Blessed L-tryptophan showed a single prominent peak at retention time (R_t) of 2.1 minutes in the LC-SM chromatograms (Figure 1). This results indicated that the sample is pure and polarity of both the samples was similar to each other. The mass spectra of both the control and Biofield Energy Treated/Blessed samples of L-tryptophan are presented in Figure 2. The mass spectra of both the samples at R_t of 2.1 minutes exhibited the presence of the molecular ion of L-tryptophan adduct with hydrogen ion at *m/z* 205.08 (calcd for $C_{11}H_{13}N_2O_2^+$, 205.1), along with low molecular fragmented mass peaks at *m/z* 188, 159, and 102 for $C_{11}H_{12}N_2O^{2+}$, $C_{10}H_{11}N_2^+$, and $C_8H_6^+$, respectively were observed both in the control and Biofield Energy Treated/Blessed L-tryptophan (Figures 3). The fragmentation pattern of the experimental data was well supported by the published literature data [41].

Isotopic Abundance Ratio Analysis

The control and Biofield Energy Treated/Blessed L-tryptophan samples showed the mass of a molecular ion at *m/z* 205.08 (calcd for $C_{11}H_{13}N_2O_2^+$, 205.1) with 100% relative abundance in the spectra. The theoretical calculation of isotopic peak P_{M+1} for the protonated L-tryptophan presented as below:

P (^{13}C) = [(11 x 1.1%) x 100% (the actual size of the M^+ peak)] / 100% = 12.1%

P (^{2}H) = [(13 x 0.015%) x 100%] / 100%= 0.195%

P (^{15}N) = [(2 x 0.4%) x 100%] / 100% = 0.8%

P (^{17}O) = [(2 x 0.04%) x 100%] / 100% = 0.08%

P_{M+1} *i.e.* ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $C_{11}H_{13}N_2O_2^+$ to *m/z* 206.08 = 13.18%

The calculated isotopic abundance of P_{M+1} value 13.18% was near to the experimental values (Table 1). From the above calculation, it has been found that ^{13}C and ^{15}N have the major contribution to *m/z* 206.08.

The LC-MS based isotopic abundance ratio analysis P_M and P_{M+1} for L-tryptophan near *m/z* 205.08 and 206.08, respectively of the control and Biofield Energy Treated samples, which were obtained from the observed relative peak intensities of $[M^+]$ and $[(M+1)^+]$ peaks, respectively in the mass spectra (Table 1). The isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in Consciousness Energy Healing Treated L-tryptophan was significantly increased by 35% compared to the control sample (Table 1). Thus, the ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $C_{11}H_{13}N_2O_2^+$ to *m/z* 206.08 in the Biofield Energy Treated sample was significantly increased compared to the control sample.

LC-MS study confirmed the structure of the sample as L-tryptophan. The isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in the Biofield Energy Treated L-tryptophan was significantly increased compared to the control sample. The Trivedi Effect®-Consciousness Energy Healing Treatment might have the impact on nuclei of L-tryptophan possibly *via* the mediation of neutrino particles would be the solid cause behind the change in the isotopic abundance ratio [15, 33, 34]. A neutrino is an elementary particle that interacts only *via* the weak subatomic force and gravity. The properties to change identities which are only possible if the neutrinos possess mass and have the ability to interchange their phase from one phase to another internally. Therefore, the neutrinos have the ability to interact with protons and neutrons in the nucleus, which indicated a close relation between neutrino and the isotope formation [36, 37]. The altered isotopic composition in molecular level of The Trivedi Effect®-Consciousness Energy Healing Treated L-tryptophan might have altered the neutron to proton ratio in the nucleus. The Biofield Energy Treated L-tryptophan with increased stable isotopic abundance ratio, might have changed the physicochemical properties with higher force constant with the atoms of the L-tryptophan. The Biofield Energy Treated L-tryptophan with improved physicochemical properties would be more desirable for the supplements, nutraceutical, and pharmaceutical formulations, which would be advantageous for the prevention and treatment of pellagra, depression, kynurenine. It could also maintain the normal label of tryptophan and avoid increase of its metabolite, lower the neurotoxin and a metabotoxin behavior, glutaric aciduria type I (glutaric acidemia type I) disorder, eosinophilia-myalgia syndrome (EMS), incurable and

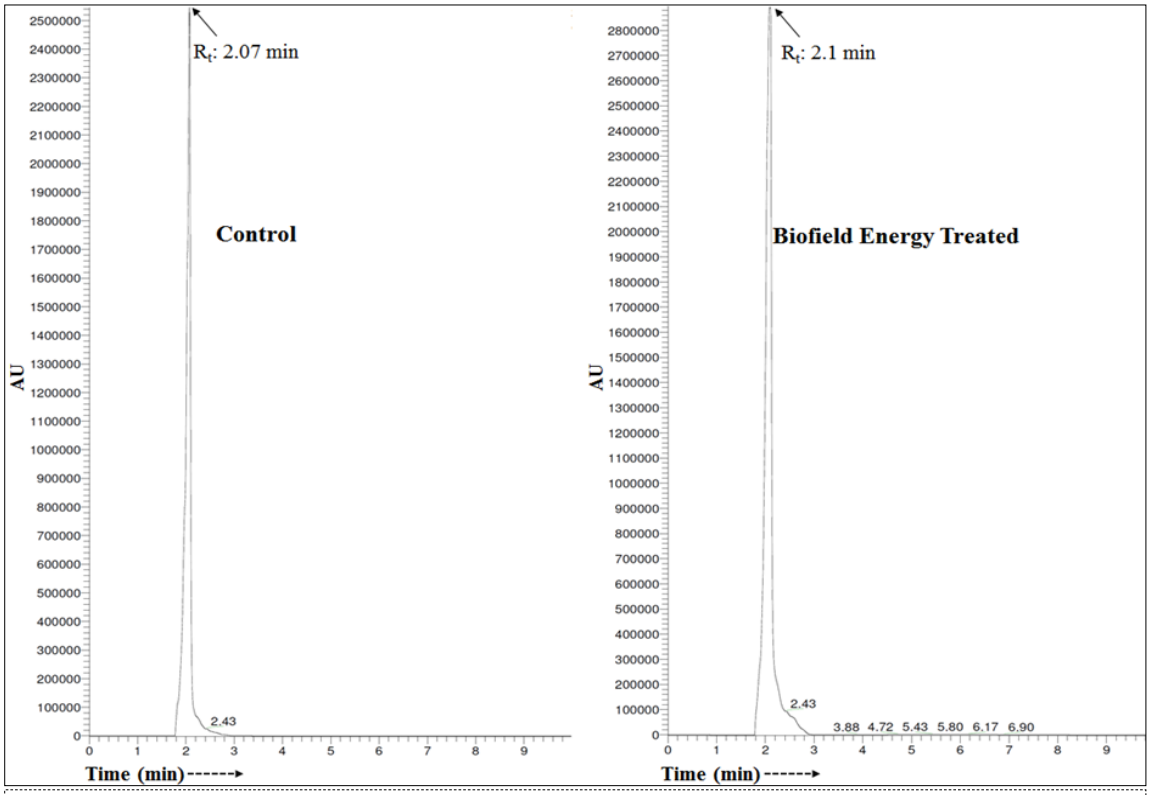

Figure 1. Liquid chromatograms of the control and Biofield Energy Treated L-tryptophan.

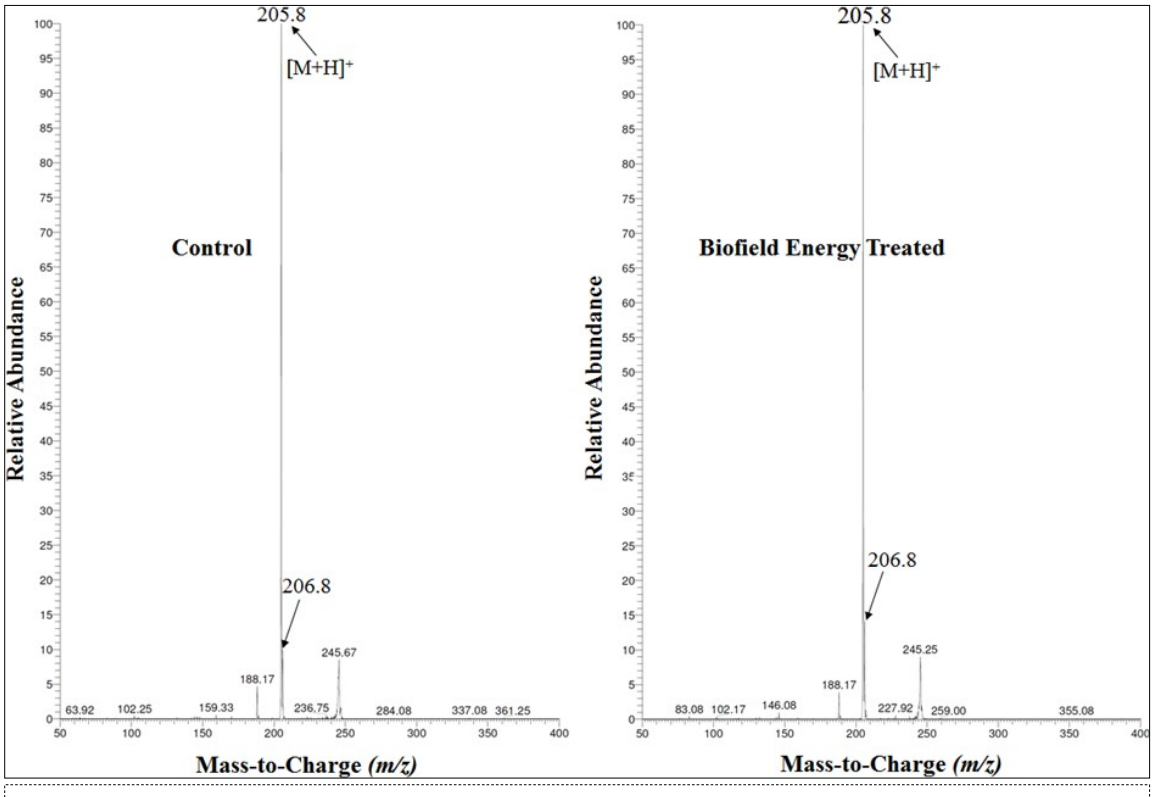

Figure 2. Mass spectra of the control and Biofield Energy Treated L-tryptophan at R_t 2.1 minutes.

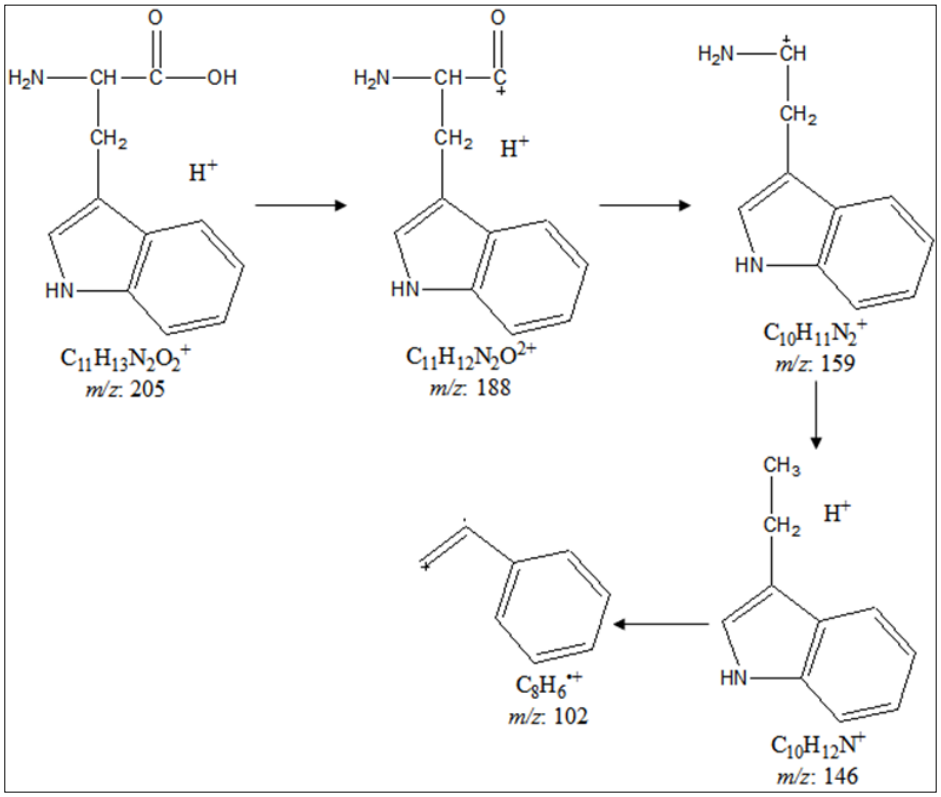

Figure 3. Proposed fragmentation pattern of L-tryptophan.

Table 1. LC-MS based isotopic abundance analysis results in Biofield Energy Treated L-tryptophan compared to the control sample.

Parameter	Control Sample	Biofield Energy Treated Sample
P_M at *m/z* 205.08 (%)	100	100
P_{M+1} at *m/z* 206.08 (%)	9.74	13.24
P_{M+1}/P_M	0.0974	0.1324
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		35.93

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.

sometimes fatal flu-like neurological condition, etc. Tryptophan is the precursor for the plant hormones like indole alkaloids and auxins. Therefore, this Biofield Energy Treated/Blessed L-tryptophan would be advantageous for the improvement of yield, productivity, and quality of crops.

Conclusions

The Trivedi Effect®-Consciousness Energy Healing Treatment/Blessing (Biofield Energy Treatment) showed the substantial impact on the isotopic abundance ratio of L-tryptophan. The LC-MS spectra of both the control and Biofield Energy Treated/Blessed samples with respect to the chromatographic peak at retention time (R_t) 2.1 minutes exhibited the mass of the molecular ion peak adduct with hydrogen ion at *m/z* 205.08 (calcd for $C_{11}H_{13}N_2O_2^+$, 205.1), along with low molecular fragmented mass peaks at *m/z* 188, 159, and 102 for $C_{11}H_{12}N_2O^{2+}$, $C_{10}H_{11}N_2^+$, and $C_8H_6^+$, respectively were also observed. The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in the Biofield Energy Treated/Blessed L-tryptophan was significantly increased by 35.93% compared with the control sample. Hence, the ^{13}C, ^2H, ^{15}N, and ^{17}O contributions from $C_{11}H_{13}N_2O_2^+$ to *m/z* 206.08 in the Biofield Energy Treated/Blessed L-tryptophan was significantly increased compared to the control sample. It could be hypothesized that the changes in the isotopic abundance and mass peak intensities due to the modification in nuclei possibly through the interference of neutrino particles using the Trivedi Effect®-Consciousness Energy Healing Treatment. The Biofield Energy Treated/Blessed L-tryptophan with increased stable isotopic abundance ratio, might have changed the physicochemical properties with higher force constant in the molecule. The new form of Biofield Energy Treated/Blessed L-tryptophan would be a better and more stable in the supplements, nutraceutical, and pharmaceutical formulations, which would be advantageous for the prevention and treatment of pellagra, depression, kynurenine. It could also maintain the normal label of tryptophan and avoid increase of its metabolite, lower the neurotoxin and a metabotoxin behavior, glutaric aciduria type I (glutaric acidemia type I) disorder, eosinophilia-myalgia syndrome (EMS), incurable and sometimes fatal flu-like neurological condition, etc. As tryptophan is the precursor for the plant hormones like indole alkaloids and auxins, hence, this Biofield Energy Treated/Blessed L-tryptophan would be advantageous for the improvement of yield, productivity, and quality of crops and other plants.

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