



The Influence of Energy of Consciousness Healing Treatment on Low Bioavailable Resveratrol in Male Sprague Dawley Rats

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To cite this article:

Alice Branton, Snehasis Jana. The Influence of Energy of Consciousness Healing Treatment on Low Bioavailable Resveratrol in Male Sprague Dawley Rats. *International Journal of Clinical and Developmental Anatomy*. Vol. 3, No. 3, 2017, pp. 9-15.

doi: 10.11648/j.ijcda.20170303.11

Received: May 20, 2017; Accepted: June 14, 2017; Published: July 14, 2017

Abstract: Resveratrol is a natural dietary antioxidant polyphenol that believed to be effective in improving overall health. The biological activity of resveratrol is limited by its poor absorption and first-pass metabolism that lead to have very low plasma concentrations following oral administration. Therefore, the present study was performed to determine the effects of The Trivedi Effect[®]-Energy of Consciousness Healing Treatment by a renowned Biofield Energy Healer, Alice Branton on resveratrol and rats through the measurement of plasma concentrations after oral administration of resveratrol. The test item, resveratrol was divided into two parts. One part was denoted as the control, while the other part was defined as the Biofield Energy Treated sample. Additionally, one group of animals also received Biofield Energy Treatment under similar conditions. Resveratrol oral formulations were administered by oral gavage at a dose of 150 mg/kg in groups *viz.* G1 (untreated resveratrol), G2 (Biofield Treated resveratrol) and G3 (Biofield Treated animals received untreated resveratrol) group. The results showed that resveratrol had a very low oral plasma exposure of 91.71 ng/mL in control group. The Biofield Treatment significantly enhanced the relative oral exposure (AUC_{0-t}) of resveratrol by 23.35% in G2 group compared to the control group. The Biofield Treatment also improved plasma peak concentration (C_{max}) of resveratrol by 125% and 28.5% in G2 and G3 groups, respectively compared with the control group. Plasma concentrations of resveratrol in G1 was declined with a rapid elimination half-life (T_{1/2}, 1.48 hours), followed by sudden increases in plasma concentrations 12 to 24 hours after the test item administration. These plasma concentrations resulted in a significant prolongation of the terminal elimination half-life of resveratrol. The oral elimination T_{1/2} of resveratrol in G2 and G3 were 4.67 and 9.0 hours, respectively as compared to the G1. The apparent oral plasma clearance of resveratrol decreased significantly by 54%, and 17.5% in G2 group and G3 group, respectively as compared to the control group. The mean residence time (MRT_{last}) of resveratrol significantly increased in G2 group (6.45 hours) and G3 group (7.70 hours), as compared to the control group. These data demonstrates greater bioavailability and total plasma level of resveratrol in rats which might be translated into better *in vivo* biological activity. Hence, The Trivedi Effect[®]-Energy of Consciousness Healing Treatment could be considered as an innovative strategy which opens new avenues to overcome poorly absorbed nutraceuticals/pharmaceuticals and can also improve the therapeutic performance of orally active molecules.

Keywords: Resveratrol, Biofield Energy Healing Treatment, Pharmacokinetics, Bioavailability, LC-MS/MS, Rat

1. Introduction

Resveratrol (3,5,4'-Trihydroxystilbene), a natural polyphenol found in some plants and fruits, has been used as

traditional medicine for over 2000 years against a wide range of biological activities. Some commonly used plants of human diets that contain a high concentration of resveratrol are blueberries (*Vaccinium* spp.), blackberries (*Morus* spp.),

and peanuts (*Arachis hypogaea*) [1, 2]. In addition, red wine made from grapes (*Vitaceae*) are considered as rich source of resveratrol in the Mediterranean diet particularly from skin, seeds, petioles, and woody parts [3]. Due to this reason, most parts of the grape plants are used for red wine production during concentration, which made it richer source of resveratrol as compared with the white wine [4, 5]. Due to its significant natural biological importance such as strong antioxidant potential, antitumor, cardiovascular, etc. properties resveratrol can be consumed *via* natural products or in the form of nutraceuticals. Among these wide pharmacological properties, some major extensively studies have been reported in humans and animal models, both *in vitro* and *in vivo* [11-13] as an anticancer agent, a platelet anti-aggregation agent, and an antioxidant, as well as its anti-aging, anti-fertility, anti-inflammatory, anti-allergenic, and so forth activities [6-10]. Due to its unique physicochemical property, its mechanism of action and issue of bioavailability play an important role for its biological action. Literature suggested that resveratrol bioavailability was determined by its rapid elimination *via* metabolism pathways that affect its absorption process. Besides, the low oral bioavailability of resveratrol (less than 1%) is due to the rapid first-pass metabolism; glucuronide and sulfate conjugates, which are the major metabolites reported in plasma [14]. Clinical studies data of resveratrol pharmacokinetic parameters led to a questions concerning whether high oral doses of resveratrol can achieve sufficient plasma concentration of resveratrol required to achieve the desirable activities like chemopreventive action [15]. Various traditional techniques have been reported in order to improve the bioavailability of compounds such as the use of co-solvents, amorphous forms, use of precipitation inhibitors, pH alteration, addition of surfactants, chemical modification of drug, etc. In addition, some methods such as modification in dielectric constant of solvent, hydrotrophy, micronization, application of ultrasonic waves, etc. were reported [16]. Authors have used complementary approach *i.e.* Biofield Energy Healing Treatment to resveratrol and animals in order to evaluate the alteration in bioavailability after treatment.

The human Biofield Energy, a subtle electromagnetic energy around the human body has significant capacity for various clinical benefits [17]. Various clinical reports suggested the significant use of energy medicine and its healing capacity, which is well demarcated by National Center for Complementary and Integrative Health (NCCIH) in order to promote wellness [18]. Biofield Energy Treatment leads to receive the energy by the object and respond in a useful way, while The Trivedi Effect[®] has been reported with the significant discovery in both living organisms and nonliving materials. The Trivedi Effect[®] has been described with significant transformation in the physicochemical properties of metals, chemicals, ceramics and polymers [18-22], improved agricultural crops overall productivity, yield and quality by several folds [23-24], altered antimicrobial characteristics of pathogenic microbes at genetic level [25-27], improved activity of nutraceutical compounds [28, 29],

livestock [30], and many more.

Recently, it has been reported in the literature that The Trivedi Effect[®] has the significant capability to alter the physicochemical and thermal properties of various pharmaceuticals, nutraceuticals, and organic compounds through possible intervention of neutrinos [31, 28]. The Trivedi Effect[®]-Consciousness Energy Healing Treatment would be a useful approach for the enhancement of the bioavailability of pharmaceuticals and nutraceuticals. The present study was planned to evaluate The Trivedi Effect[®]-Consciousness Energy Healing Treatment on the test item (resveratrol), and test system (rat) through the estimation of resveratrol in plasma concentration after a single dose of oral administration of resveratrol in rat.

2. Materials and Methods

2.1. Chemicals and Reagents

Resveratrol chloride and telmisartan were purchased from TCI, Japan and Sigma (St. Louis, MO, USA), respectively. The reagents used for sample preparation and bioanalysis included acetonitrile (HPLC Grade, Merck), methanol (HPLC Grade, Merck), water (Milli-Q), and formic acid (LC-MS Grade, Fluka). USP grade nitrogen was used as the curtain gas and collision gas for LC-MS/MS were supplied from air compressor (Anesta Iwata, Japan), polypropylene tubes (Tarsons, India), class-A, measuring cylinders and volumetric flasks (Borosil, Germany) and membrane filters, 0.22 μm and 0.45 μm (Millipore) were used during the study. All other reagents and solvents were of analytical grade available from India.

2.2. Energy of Consciousness Treatment Strategies

The test item, resveratrol was divided into two parts. One part was considered as the control group, while the other part was defined as the Biofield Energy Treated test group. The test item in Biofield Treated group was subjected to The Trivedi Effect[®]- Energy of Consciousness Healing Treatment by a renowned Biofield Energy Healer, Alice Branton, USA. Additionally, one group of animals also received the Biofield Energy Treatment *per se* by the same Biofield Energy Healer under similar experimental conditions. This Biofield Treatment was provided for approximately 5 minutes through the Biofield Energy Healer's unique Energy Transmission process (The Trivedi Effect[®]), administered to the test sample and animals. Similarly, the control test sample was subjected to "sham" healer for 5 minutes, under similar laboratory conditions without having any awareness about the Biofield Energy Treatment. Further, the Biofield Energy Treated and untreated test items were kept in similar sealed conditions and used for the study as per design.

2.3. In Vivo Pharmacokinetics Study

2.3.1. Animals

Male Sprague-Dawley (SD) rats (body weight 230 - 270 gm) were procured from Liveon Biosciences, Bangalore,

India. Animals were housed in polycarbonate cage. For maintenance of animals, standard conditions such as temperature and humidity were maintained at $22 \pm 3^\circ\text{C}$ and 40-70%, respectively and illumination was controlled to give a sequence of 12 hours light and 12 hours dark cycle. The temperature and humidity were recorded by auto-controlled data logger system. All the animals were provided laboratory rodent diet (Vetcare India Pvt. Ltd, Bengaluru). Reverse osmosis water treated with ultraviolet light was provided *ad libitum*. The experiments using animals in this investigation were performed in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in The Gazette of India, January 7, 2010 and protocol approved by the Institutional (GVK Bio) Animal Ethics Committee (IAEC approval number: BA-011).

2.3.2. Experimental Design

Rats were divided into three groups ($n = 3$): group 1 (Gr. 1) – per oral (*p.o.*) dosing of untreated resveratrol, group 2 (Gr. 2) – per oral (*p.o.*) dosing of Biofield Energy Treated resveratrol and group 3 (Gr. 3) – per oral (*p.o.*) dosing of untreated resveratrol in the Biofield Energy Treated animals. All animals were received per oral dose at 100 mg/kg of resveratrol solution formulation. The dose (150 mg/kg) of the test item was chosen based on the preliminary experiments performed in the laboratory and observed the quantifiable concentration of this analyte in rat plasma.

2.4. Formulation Preparation

The solution formulations of the test item was prepared in 40% *w/v* 2-Hydroxypropyl- β -cyclodextrin in distilled water. All formulations were prepared freshly prior to dosing. The dose volume for per oral route was 10 mL/kg.

2.5. Pharmacokinetic Studies

The solution of resveratrol chloride formulations were freshly prepared for per oral dosing. All rats were fasted overnight and the fasting continued up to 4 hours post dosing with free access to drinking water. The oral test formulation was administered at 150 mg/kg dose through oral gavage using an 18G stainless steel intubation cannula. The dosing volume administered was 10 mL/kg. Blood samples (~120 μL) were collected from the jugular vein catheter of three rats from each group at each time point [pre-dose, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours (*p.o.*)]. Samples were collected into labeled micro centrifuge tubes, containing 20% *w/v* K_2EDTA as an anticoagulant. Plasma samples were separated from the blood by centrifugation at 2500 *g* for 10 min at $4 \pm 2^\circ\text{C}$ and stored below -40°C (Thermo Scientific, USA) deep freezer until bioanalysis.

2.6. LC-MS/MS Analysis

LC-MS/MS analysis of rat plasma samples was performed using API 4500 Applied Biosystem-SCIEX (Concord, Ontario, Canada) triple quadrupole mass analyzer system with

the Turbo Ion Spray interface connected to a Shimadzu UFLC system (Shimadzu Corp., Japan). The optimum operating parameters were determined by electro spray ionization (ESI) interface in negative ion mode. A generic mass spectrometry parameters of the analyte were developed and used for the analysis. These parameters were the declustering potential range (-80), collision energy range (-34), collision cell exit potential range (-8), curtain gas (30 arbitrary units), collisionally activated dissociation gas (10), ionspray voltage (-4500 V), source temperature (550°C), and ion source/nebulizer gas 1 and gas 2 (50 and 55, respectively arbitrary units each). Interface heaters were kept on for the analyte. The analyte was detected by negative Turbo Ion Spray in the multiple reaction monitoring mode (MRM) mode using predetermined parent/product mass transition ion pairs. The parameters of the selected MRM monitoring transitions for the $[\text{M} - \text{H}]^-$ precursor ion to selected product ion (*m/z*) were optimized with 226.90/142.90 (resveratrol), and 515.30/286.70 (telmisartan as an internal standard). The whole system was controlled by Analyst[®] software version 1.6.3 (Applied Biosystem/MDS SCIEX, Concord, Canada). Stock solutions of resveratrol and telmisartan (internal standard, IS) were prepared in methanol at approximately 9.999 mg/mL and 0.98 mg/mL, respectively and subsequently diluted which were used for the bioanalysis.

The extraction procedure for plasma samples or the spiked into plasma calibration standards were identical. A 50 μL sample of either study sample or spiked calibration standard was added to individual pre-labeled micro-centrifuge tubes. A 50 μL sample of either study sample or spiked calibration standard/quality control samples were added to individual wells of 96 well plate with 500 μL capacity. 200 μL of internal standard (IS) prepared in acetonitrile (ACN) was added to the samples in deep well plate except for blank, where 200 μL of ACN was added and vortexed for 5 minutes. Samples were centrifuged for 10 minutes at a speed of 4000 rpm (3220 *g*) at 4°C . Following centrifugation, 120 μL of supernatant was transferred into 1000 μL capacity deep well plate and mixed with 120 μL of methanol: water, 50:50 *v/v*. The plate was kept in the auto-sampler for the LC-MS/MS analysis.

A Shimadzu LC-20AD LC system (Shimadzu Corp., Japan) was connected to a SIL -20 AC HT auto-sampler (Shimadzu Corp., Japan). The supernatant was injected (15 μL) onto a 50 x 4.6 mm (3.5 μm) Waters, X-Bridge, C18 HPLC column (Waters, Massachusetts, Ireland). Analytes were eluted using a gradient elution program with a mobile phase consists of 10 mM ammonium acetate in water (pump A) with methanol (pump B) at a flow rate of 1.0 mL/min. The column temperature was at 40°C and the sample temperature was at 15°C . The following linear gradient was employed for the separation: 95% A for 0.01 min, 60% A at 0.5 min, 40% A at 1.0 min, 25% A at 2.0 min, and hold to 3.2 min, 95% A at 3.3 min and hold to 5.0 min. The resveratrol and telmisartan elution times were approximately 1.69 and 2.22 min, respectively. Peak integration, regression and calculation of analytes concentration were computed using Analyst Classic

(Version 1.6.3) software. The calibration curve was performed by linear curve fit of the peak area ratio (analyte/internal standard) as a function of the concentration in the respective matrix. A weighting of $1/x^2$ (where x is the concentration of a given calibration standard level) was found to be optimal. The lower limit of quantification (LLOQ) in rat plasma was 1.02 ng/mL for resveratrol. Analysis of resveratrol in plasma (1.04 – 1038.96 ng/mL) showed a repeatability (relative standard deviation-RSD%) of 1.5% to 8.47% and accuracy of 100.18% to 108.81%.

2.7. Pharmacokinetic Analysis

The pharmacokinetic parameters of resveratrol were obtained by noncompartmental analysis module in Phoenix WinNonlin® (Version 7.0) (Pharsight, Mountain View, CA). The areas under the concentration time curve (AUC_{0-t} and $AUC_{0-\infty}$) were calculated by linear trapezoidal rule. The terminal elimination rate constant (k_{el}) was determined by regression analysis of the linear terminal portion of the log plasma concentration-time curve. The terminal half-life ($T_{1/2}$)

Table 1. Pharmacokinetic parameters of resveratrol after *p.o.* administration at 100 mg/kg body weight to Sprague Dawley male rats.

Parameter	Gr. 1 (Untreated Resveratrol)	Gr. 2 (Biofield Treated Resveratrol)	Gr. 3 (Biofield Treated Rats + Untreated Resveratrol)
C_{max} (ng/mL)	43.30	97.37	55.64
T_{max} (hours)	0.50	0.42	0.25
AUC_{0-t} (ng/mL*hours)	91.71	113.13	76.75
T_{half} (hours)	1.48	7.96	10.03
MRT_{last} (hours)	1.61	6.45	7.7
CL/F (L/hours/kg)	8.06	3.64	6.65
Vd/F (L/kg)	1724.75	1234.55	2328.4
K_{el} (hour ⁻¹)	0.1	0.09	0.1
K_a (hour ⁻¹)	0.4	0.31	0.21
MAT (hour)	7.09	3.63	6
% Change in Fr		23.35	16.28

The data are expressed as mean values. AUC: area under the plasma concentration–time curve from 0 hours to infinity; CL/F: apparent oral plasma clearance; Vd/F: apparent volume of distribution; C_{max} : peak concentration; T_{max} : time to reach peak concentration; $T_{1/2}$: terminal half-life; K_{el} : absorption rate constant; K_a : absorption rate constant, MRT: mean residence time; MAT: mean absorption time; *p.o.*: per oral; Fr: Relative oral Bioavailability.

The C_{max} of resveratrol formulation in control group was 43.3 ng/mL after 0.50 hours, whereas it was 97.37 ng/mL and 55.64 ng/mL for the resveratrol formulation after 0.42 hours and 0.25 hours, in G2 and G3 group, respectively. The results showed that resveratrol had a very low oral exposure (AUC_{0-t}) of 91.71 ng/mL in control (untreated) group. After the Biofield Energy Treatment by a renowned Biofield Energy Healer, Alice Branton, the relative oral exposure (AUC_{0-t}) of resveratrol was enhanced significantly by 23.36% in G2 group, as compared to the control group. The Biofield Energy Treatment also improved plasma peak concentration (C_{max}) of resveratrol by 125% and 28.5% in G2 and G3 groups, respectively as compared to the control group.

was estimated as $0.693/k_{el}$; the apparent oral clearance (CL/F) were calculated for per oral dose divided by AUC, respectively. Peak resveratrol concentrations (C_{max}) and the times when they occurred (t_{max}) were derived directly from the data. The relative oral bioavailability (Fr) was estimated by $AUC_{treated}/AUC_{control}$.

2.8. Statistical Analysis

All mean values are presented with their standard deviation (mean \pm S.D.). Data were analyzed for statistically significant differences using analysis of variance followed by the two-sided unpaired Student's *t*-test. Differences were considered to be significant at a level of $p < 0.05$.

3. Results and Discussions

The mean pharmacokinetic parameters and profiles of resveratrol in the rat plasma after a single oral dose of administration of solution formulations in three different groups are summarized in Table 1 and Figure 1, respectively.

After oral administration, plasma concentrations of resveratrol in control group (G1) declined with a rapid elimination half-life ($T_{1/2}$, 1.48 hours), followed by sudden increases in plasma concentrations at 12 hours and 24 hours after the test item administration (Figure 1). These plasma concentrations resulted in a significant prolongation of the terminal elimination half-life of resveratrol. The oral elimination half-life ($T_{1/2}$) of resveratrol in the Biofield Energy Treatment group (G2) and Biofield Treated rat group (G3) were 4.67 and 9.0 hours, reactively as compared to the control group (G1). The apparent oral plasma clearance of resveratrol was decreased significantly ($p < 0.05$) by 54.58%, and 17.5% in G2 group and G3 group, respectively as compared to the control group. The mean residence time (MRT_{last}) of resveratrol was significantly increased in G2 group (6.45 hours) and G3 group (7.70 hours), as compared to the control group. These data demonstrates greater bioavailability and total plasma level of resveratrol compound in rats which might be translated into better *in vivo* biological activity of resveratrol.

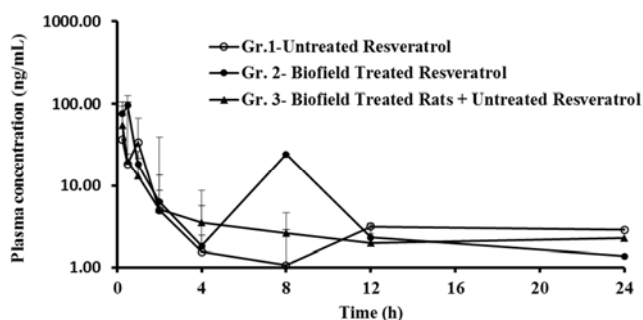


Figure 1. Mean plasma concentration–time profiles of resveratrol after per oral (p.o.) administration (100 mg/kg) to Sprague Dawley male rats. The data are expressed as mean \pm S.D (n = 3).

The results indicated that the Biofield Energy Treated resveratrol and animals *per se* significantly increased the rate and extent of oral absorption of resveratrol. The enhanced absorption efficiency may be explained as follows: (1) the huge specific surface area of the resveratrol formulation, (2) the stability of the resveratrol formulation in the gastrointestinal tract, and (3) delayed resveratrol metabolism pathways. The significant improvement of relative oral bioavailability of resveratrol in Biofield Energy Treated group might be translated into increased pharmacological effects in various disease conditions.

Resveratrol is a natural polyphenol with a stilbene structure containing two phenolic rings bonded together by a double styrene bond that results in 3,5,4'-trihydroxystilbene (molecular weight 228.25 g/mol) [32]. The isometric *cis*- and *trans*-forms of resveratrol is due to the double bond present in the structure. The accumulation of resveratrol in plants is produced by a mechanism of resistance to parasites and other adverse conditions such as chemical substances, fungal infection, UV radiation, and in general, stressful factors for the plant [33-35]. Literature data suggest that most of the plant species produced resveratrol in response to stressful conditions [36] and was found in most of the fruits of human diets [37, 38]. *Cis*- and *trans*-isomers co-occur in plants and in wine, while grape extract lacks the *cis*-resveratrol form [39]. However, more predominant and stable natural form of resveratrol is *trans*-isomer. Resveratrol has wide biological importance such as reduction of cardiovascular risk [40], minimize the incidence of arterial hypertension, heart failure and ischemic cardiac disease [41, 42], improve insulin sensitivity by minimizing the plasma glycemia levels, and obesity in rodent models [43]. Due to its different physicochemical properties such as molecular weight, number of hydrogen bond donor and acceptor, rotatable bond, clogP, and polar surface area, resveratrol has poor bio-membrane permeability. The poor oral bioavailability [14] considerably less than 1% would attribute to different the metabolic issue [44], such as very short half-life in rat liver microsomes. Furthermore, poor oral bioavailability is due to the first-pass metabolism in intestine [45, 46], while increased dose and repeated dose escalation would not affect the bioavailability. Therefore, this study was carried out to evaluate the pharmacokinetic properties of resveratrol using

the novel technique known as The Trivedi Effect[®]-Energy of Consciousness Healing Treatment on resveratrol and animals. To the authors' knowledge, this is the first report to demonstrate the effects of Biofield Energy Treatment on resveratrol pharmacokinetics in rats after a single dose of oral administration. Pharmacokinetic profiles of resveratrol in three different groups were compared in male rats following a single oral (gavage) dose. The study results demonstrated that markedly higher C_{max} values for the Biofield Energy Treated groups (G2 and G3) were observed as compared to untreated groups (G1). The relative oral bioavailability of Biofield Treated group (G2) was significantly higher than the oral bioavailability of control group. It also found that oral plasma clearance (CL/F) and $T_{1/2}$ values of resveratrol were significantly altered in the Biofield Treated groups. Lower clearance values in G2 group was correlated well for the higher plasma exposure (AUC_{0-t}) to resveratrol in comparison with the untreated group. The results clearly demonstrate the significant effect of Biofield Energy Treatment with respect to the increased oral exposure of resveratrol.

4. Conclusions

The Trivedi Effect[®]-Energy of Consciousness Healing Treatment significantly enhanced the relative oral exposure (AUC_{0-t}) of resveratrol by 23.35% in the G2 group compared to the control group. The Biofield Energy Treatment also improved the plasma peak concentration (C_{max}) of resveratrol by 125% and 28.5% in the G2 and G3 group, respectively as compared to the control group. The oral elimination half-life ($T_{1/2}$) of resveratrol in the Biofield Energy Treatment group (G2) and Biofield Treated animal group *per se* (G3) were 4.67 and 9.0 hours, respectively as compared to the control group (G1). The apparent oral plasma clearance of resveratrol was decreased significantly ($p < 0.05$) by 54% and 17.5% in the G2 and G3 group, respectively as compared to the control group. The mean residence time (MRT_{last}) of resveratrol was significantly increased in G2 group (6.45 hours) and G3 group (7.70 hours), as compared to the control group. These data demonstrates greater bioavailability and total plasma level of resveratrol observed in rats which might be translated into better *in vivo* biological activity of resveratrol. An improvement of oral resveratrol bioavailability might be due to the alteration of physicochemical properties and thermal properties by Biofield Energy Treatment. The Energy of Consciousness Healing Treatment is shown here as an innovative strategy which opens new avenues to overcome poorly absorbed drug/nutraceuticals/herbal extracts and can also improve the therapeutic performance of orally active molecules. Thereby, the Biofield Energy Treated resveratrol and Biofield Energy Healing Treatment *per se* showed improved bioavailability, which can significantly prevent or delay the onset of cancer, heart disease, neurodegenerative diseases, metabolic disorders, ischaemic and chemically induced injuries, diabetes,

pathological inflammation, and viral infection.

Acknowledgements

Authors are grateful to GVK Bioscience, Trivedi Science, Trivedi Global, Inc., Trivedi Testimonials and Trivedi Master Wellness for their support throughout the work.

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