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The Structure and Function of Horseradish Peroxidase (HRP) under the Influence of Faradarmani Consciousness Field

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Abstract

The study of biological enzymes, with their unique characteristics such as substrate interactions, structural changes, and catalytic rates, plays a key role in both basic and applied enzymology research. Horseradish peroxidase (HRP) is among the enzymes that have been repeatedly used to study the metabolism of biological systems. The Faradarmani Consciousness Field is non-frequency in nature, and its effect is initiated through the mind of an announcer, referred to as a Faradarmangar (a trained individual). In this study, the effect of this field on the structure and activity of HRP was examined in two forms: the commercially purchased sample and the sample extracted from the plant. This design allows us not only to record the effects of the Faradarmani Consciousness Field but also to replicate and examine changes in the maximum velocity and Michaelis constant under different conditions. The results showed that in the commercial sample, the Faradarmani Consciousness Field decreased the maximum velocity by 5% compared to the control and increased the Michaelis constant by 18%. In contrast, the plant-extracted samples showed increases of 6% and 40% in maximum velocity and Michaelis constant, respectively, compared to the control under the influence of the Faradarmani Consciousness Field. Changes in fluorescence emission intensity were also observed, suggesting structural alterations. Further studies are needed to clarify the interaction between this non-frequency field and biological enzymes.

Keywords: Faradarmani Consciousness Field (FCF); horseradish peroxidase; Michaelis constant; maximum velocity

Introduction

Oxidoreductases are a superfamily of peroxidases (POXs) (EC 1.11.1.7) capable of catalyzing the oxidation of a wide variety of organic and inorganic substrates using hydrogen peroxide (H_2O_2) (Conesa et al., 2002). HRP is a heme-containing enzyme obtained from horseradish roots, which is absent from most mammalian cells. Our understanding of the structure and function of HRP has been achieved by X-ray crystallography (Gajhede et al., 1997) and recombinant enzymes (Smith et al., 1990). HRP has been the subject of much research in the fields of medicine, life sciences, biotechnology, therapeutics, biosensor systems, etc. (Krainer et al., 2015). In plants, HRP isoenzymes contribute to indole-3-acetic acid metabolism, lignification, cross-linking of cell wall polymers, suberin formation, and resistance to infection (Veitch, 2004).

Reactive oxygen species (ROS) are typically produced as a product of cellular metabolism pathways, but their levels increase under environmental stresses. These free radicals destroy cellular structures and induce apoptosis, or programmed cell death. It has been found that enzymes play an important protective role against oxidative stress and in the prevention of apoptosis (Khavari-Nejad et al., 2015).

Additionally, POXs have been shown to increase in activity upon pathogen infection, such as fungi (Harrison et al., 1995). There are many recorded reports demonstrating that the increased levels of antioxidant enzyme activity in plants result in stronger resistance against environmental stresses (Demiral and Turkan, 2005). On the other hand, endogenous ROS such as H_2O_2 have been regarded as a messenger molecule, which contributes to various vital processes including proliferation, differentiation, tissue repair, inflammation, aging, etc. (Sies, 2014).

The Michaelis constant (Km) and the maximum velocity (Vmax), which are the building blocks of the Michaelis-Menten (MM) equation, are considered the functional constants of enzymes. These constants, along with other kinetic parameters derived from their ratios—such as the turnover number (kcat) and the specificity constant (kcat/Km)—reflect the catalytic efficiency of enzymes at defined substrate and enzyme concentrations. The MM equation has been used in studies to investigate the structure and function of various enzymes under different environmental conditions, such as temperature and buffer types (Bisswanger, 1994), various concentrations of inhibitors and inducers (Lopina, 2017), and comparisons of the kinetic properties of wild-type and mutant enzymes (Humer and Spadiut, 2019).

One of the topics of interest during the 1970s and 1980s was examining the values of these constants and their changes during the evolution of enzymes. Some studies have shown decreases and increases in Km in parallel with kcat and Vmax during evolution (Fersht, 1974), as well as their heritable changes due to adaptation (Crowley, 1975). It has been reported that in many organisms, kinetic mechanisms have evolved to aid survival in response to changing environmental factors (Ulusu, 2015).

All the aforementioned studies in the field of enzyme kinetics have focused on the effects of various physicochemical and environmental factors on enzymatic constants. However, to date, no study has investigated the potential impact of mind–body methods or consciousness-related interactions on enzymatic activity and function.

According to Taheri's theories, there are various T-Consciousness Fields, which are subsets of the Cosmic Consciousness Network. These fields do not have a frequency-based or physical nature. Therefore, they cannot be measured directly using quantitative instruments. However, it is possible to record the effects of these fields in the laboratory by applying them to different subjects, including biological samples or inanimate materials (Taheri, 2013).

The effects of the T-Consciousness Fields are initiated through the mind of an announcer, referred to as a Faradarmangar (a trained individual), and are initiated with a brief and instantaneous attention to the subject under study. This characteristic has motivated researchers to design various experiments in this regard and report the resulting findings. In previous studies on wheat, we observed that

the Faradarmani Consciousness Field reduced the harmful effects of salt stress on wheat by inducing the activity of antioxidant enzymes (Torabi et al., 2020). The effects of this field have also been reported on the electrical activity of Faradarmangar brains (Taheri et al., 2020), the MCF7 cancer cell line (Taheri et al., 2020a), *in vitro* and *in vivo* models of Alzheimer's disease (Taheri et al., 2021b, Taheri et al., 2021c), and microorganisms (Taheri et al., 2021d, Taheri et al., 2022). The aim of the present study was to investigate the effects of the Faradarmani Consciousness Field on the activity and structure of HRP enzyme in both commercially obtained and plant-extracted samples.

Materials and Methods

In this study, we used HRP obtained from two sources. The first is the commercial HRP, manufactured and sold under the brand [Sigma], and the second is HRP extracted from the plant (horseradish).

This diversity in the sources gives us the possibility of reproducibility of the FCF treatment at multiple times and stages. For example, the Faradarmani Consciousness Field was applied during the extraction, afterward during the enzymatic kinetic assay, or at both stages. Respective Materials and Methods sections for each source are provided separately.

Application of Faradarmani Consciousness Field

The Faradarmani Consciousness Field was applied to the samples according to the protocol established by the Cosmointel Research Center1[1]. Further details are provided in the general considerations of this issue.

Commercial HRP Materials

HRP (donor: HRP, oxidoreductase, EC 1.11.1.7) and 3,3',5,5'-Tetramethylbenzidine (TMB) were purchased from Sigma Co. Ltd. Other reagents and chemicals were of analytical reagent grade. Double-distilled water was used throughout the experiments.

Methods for commerical HRP

The UV/Vis spectra were recorded on a Varian Cary Bio 100 spectrophotometer. Intrinsic fluorescence spectra of the protein were measured at 25°C using a Varian Cary Eclipse fluorescence spectrophotometer with excitation and emission slit widths of five nm. Fluorescence emission from Trp was measured using excitation at 295 nm to avoid the contribution of tyrosine. In intrinsic fluorescence studies, the protein concentration was 90.4 µM in 0.2 M potassium phosphate buffer, pH 7.0.

Enzyme Assay

Freshly prepared enzyme solutions were used throughout the experiments. The enzyme stock solution was prepared by dissolving HRP (1.5 mg) in PBS (3 mL, 0.01 M, pH 7) and kept on ice. The stock solution of H_2O_2 (0.1 M) was prepared by diluting H_2O_2 (0.5 mL, 30 wt%) to 50 mL in PBS (0.01 M, pH 7). The HRP enzymatic reactions were carried out in PBS (0.01 M, pH 7) at 20°C. All enzymatic reactions were conducted in conventional quartz UV-visible cuvettes in sets of three replicates. The total volume of the reaction mixture was 1.5 mL. Experiments were carried out in the presence of a constant concentration of HRP (50 µL) and H_2O_2 (10 µL) with different concentrations of TMB (1.23, 1.85, 3.7, 7.4, 11.1, and 14.8 mM). The colorimetric changes were determined by recording the absorbance at 652 nm.

Kinetic Parameters

The kinetic constants Vmax and Km were calculated by fitting the reaction velocity values and substrate concentrations to the Michaelis–Menten equation as follows:

$$V = (Vmax \times [S]) / (Km + [S])$$

Where V is the initial reaction velocity, Vmax is the maximal reaction rate observed at saturating substrate concentrations, [S] is the substrate concentration, and Km is the Michaelis constant. In all steps, two containers were used as the blank and the sample, each with different labels.

1.www.cosmointel.com

Extracted HRP Materials

3,3',5,5'-Tetramethylbenzidine (TMB) was purchased from Sigma Co. Ltd. Horseradish roots were obtained from local markets. Other reagents and chemicals were of analytical reagent grade. Double-distilled water was used throughout the experiments.

Methods

Enzyme extraction from horseradish roots

About 100 g of horseradish was thoroughly washed with water, cut into small pieces, and homogenized in a blender with 500 mL of distilled water. The mixture was centrifuged at 10,000 rpm for 5 min at 4°C. The filtrate was heated in a water bath at 65°C for three minutes to inactivate catalases and then cooled quickly in ice water. The enzyme was precipitated with ammonium sulfate for partial purification. Solid ammonium sulfate was added to 100 mL of peroxidase at a concentration of 30% (w/v). The suspension was stirred for half an hour at 4°C. After sufficient mixing, the precipitates were collected by centrifugation at 10,000 rpm for 30 min. Enzyme activity was determined for each concentration, and the precipitates were collected for further purification.

Dialysis

Before the dialysis procedure, the dialysis bag was boiled in 0.1 M sodium carbonate solution for 1 hour and then left overnight. This procedure allows the dialysis bag to open properly. The precipitates obtained by ammonium sulfate precipitation were dialyzed in a dialysis bag against 0.2 M phosphate buffer (pH 6.5) with constant stirring on a magnetic stirrer for 2 hours. The dialyzed sample was then subjected to enzyme assay and protein estimation.

Protein estimation

Proteins were estimated according to the method of Lowry (1957). Solution A was prepared by dissolving sodium carbonate in distilled water and adjusting the volume to 500 mL. Solution B consisted of 0.38 g $CuSO_4 \cdot 5H_2O$ and 0.6 g potassium–sodium tartrate dissolved in distilled water to a final volume of 500 mL. Solution C was the Folin reagent without dilution. Solution D consisted of 1 N NaOH.

Spectrophotometric Procedures

The UV/Vis spectra were recorded on a Varian Cary Bio 100 spectrophotometer. Intrinsic fluorescence spectra of the protein were measured at 25˚C using a Varian Cary Eclipse fluorescence spectrophotometer with the excitation and emission slit widths of 5 nm. Fluorescence emission from Trp was measured using excitation at 295 nm to avoid the contribution of tyrosine. In intrinsic fluorescence studies, the concentration of protein was 45.2 μM in 0.2 M potassium phosphate buffer, pH 7.0.

The sections related to enzyme assay, and the calculation of kinetic parameters were performed in the same manner as described in the previous section.

Statistical Analysis

In this study, each sample had three replicates, and no data were excluded. Comparisons between the test and control samples were analyzed using the t-test in SPSS software, version 17, and a confidence level of 95% was considered statistically significant.

Results

Figure 1 shows the changes in the Lineweaver–Burk plots for samples with and without the Faradarmani Consciousness Field (control) for commercial HRP. The kinetic parameters of the HRP are presented in Table 1 based on the data from Figure 1. As observed, the Faradarmani Consciousness Field caused a 4.8% decrease in Vmax and an 18.2% increase in Km of the commercial HRP during the kinetic assay. In addition, the catalytic efficiency and the turnover and specificity constant of the HRP under the influence of this field decreased by approximately 5% and 19%, respectively.

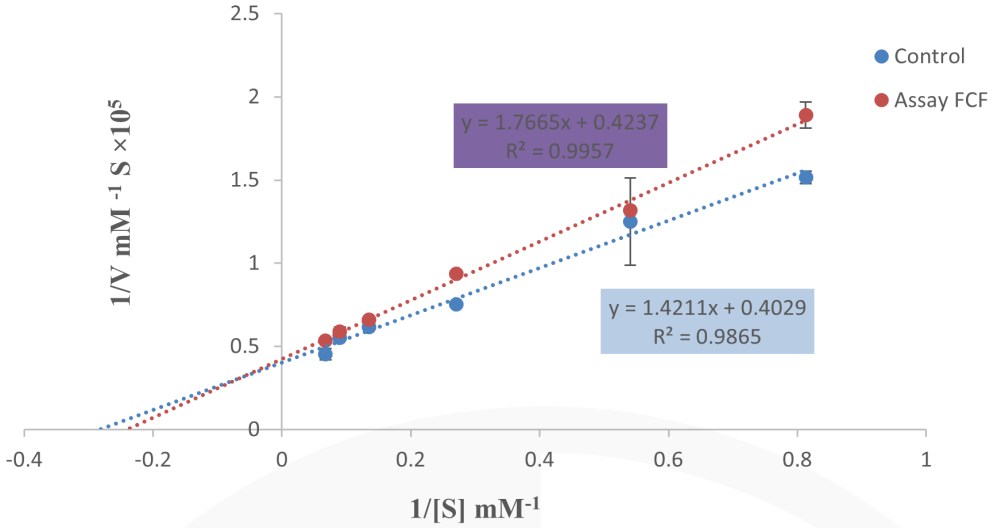


Figure 1. Changes in the Lineweaver–Burk plot of commercial HRP enzyme in samples with the Faradarmani Consciousness Field (FCF) and control.

Table 1. Calculated kinetic constants for the commercial HRP sample in the control and under the influence of the Faradarmani Consciousness Field (FCF).

	Vmax (mM/s) × 10^{-5}	Km (mM)	kcat (s^{-1})	kcat/Km ($M^{-1}s^{-1}$)	% Δ Vmax	% Δ Km	% Δ kcat	% Δ kcat/ Km
Control	2.48	3.52	0.0620	17.6	-	-	-	-
FCF	2.36	4.16	0.0589	14.2	-4.8%	18.2%	-5.0%	-19.3%

Figure 2 shows the changes in the fluorescence emission of commercial HRP in the control samples and those under the Faradarmani Consciousness Field. As observed, the field caused a 4% decrease in fluorescence emission.

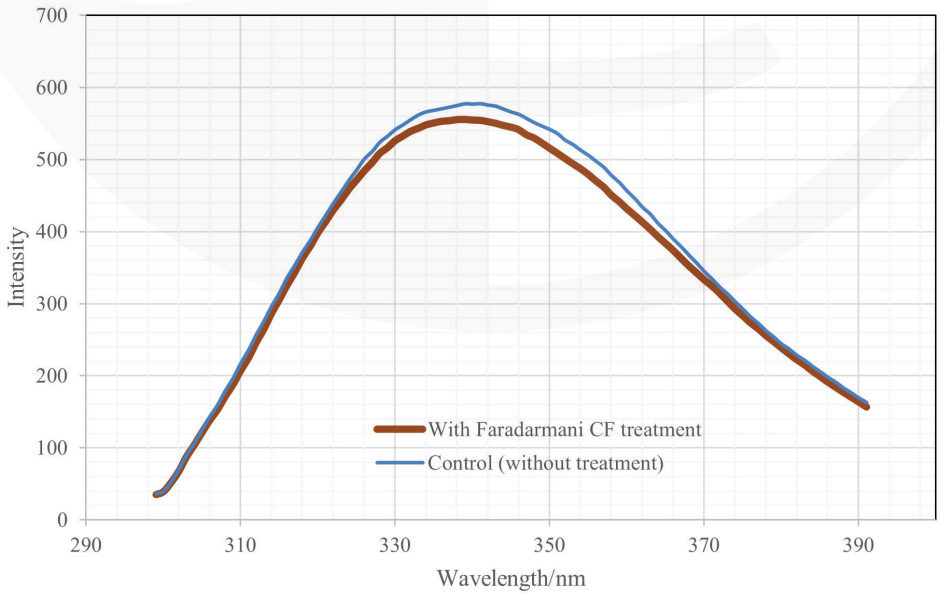


Figure 2. Fluorescence emission of the commercial HRP sample under Faradarmani Consciousness Field treatment compared to the control.

Results of the Extracted Plant HRP Analysis

Since the Faradarmani Consciousness Field was applied at three stages—during extraction, kinetic measurement, or at both stages—as shown in Table 2, four types of data presentation were provided considering the control samples (without the field effect), and the values of maximum velocity (Vmax) and Michaelis constant (Km) were reported. These values were determined using the Lineweaver–Burk plot (Figure 3).

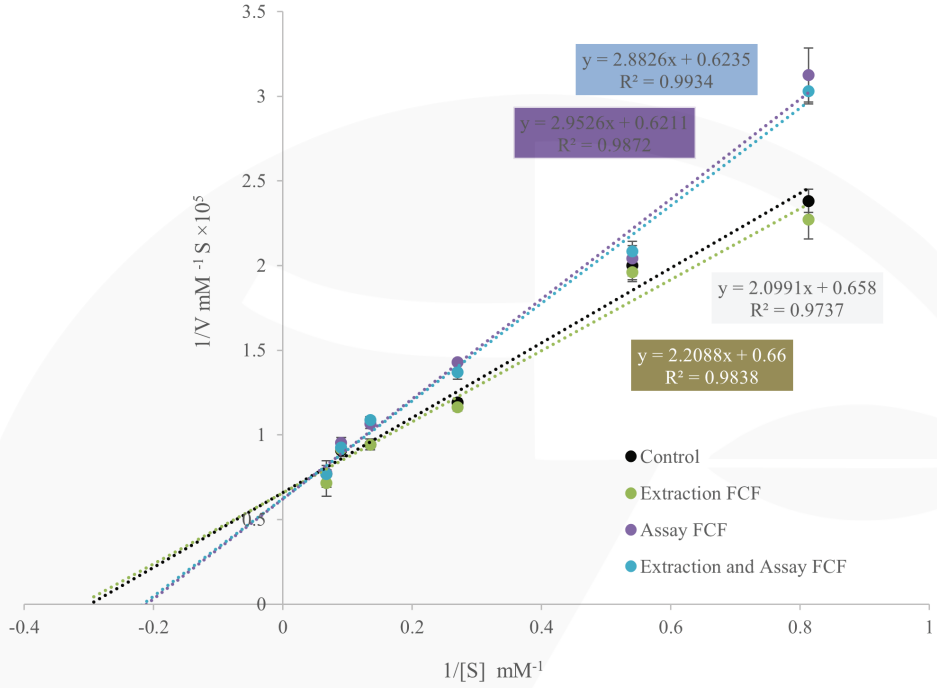


Figure 3. Changes in the Lineweaver–Burk plot of plant-extracted HRP enzyme in control samples and in samples exposed to the Faradarmani Consciousness Field at different stages, including extraction, kinetic assay, or both stages.

As shown in Table 2, when the Faradarmani Consciousness Field was applied only during the extraction stage, Vmax and Kcat increased slightly by approximately 0.7% and 0.5%, respectively, while Km decreased by about 4.5%. When the field was applied only during the kinetic assay, greater increases in Vmax and Kcat were observed, approximately 6.6% and 6.9%, respectively. However, Faradarmani treatment led to a significant 42.5% increase in Km, resulting in an approximately 25.2% decrease in the Kcat/Km ratio. The data for both extraction and assay stages were similar to those observed for the assay stage alone.

Table 2. Kinetic constants for plant-extracted HRP samples based on Lineweaver–Burk analysis in the control and at different stages of Faradarmani Consciousness Field (FCF) application.

Test No.	FCF Treatment	Vmax (mM/s) × 10^{-5}	Km (mM)	kcat	kcat/Km	% Δ Vmax	% Δ Km	% Δ kcat	% Δ kcat/Km
1	Control	1.51	3.34	0.0377	11.3	-	-	-	-
2	Extraction + FCF	1.52	3.19	0.0379	11.9	0.7%	-4.5%	0.5%	5.3%
3	Assay + FCF	1.61	4.76	0.0403	8.45	6.6%	42.5%	6.9%	-25.2%
4	Extraction & Assay + FCF	1.60	4.62	0.0400	8.65	6.0%	38.3%	6.1%	-23.5%

Figure 4 shows the fluorescence intensity of the extracted enzyme under Faradarmani Consciousness Field treatment compared to the control (extracted enzyme without treatment), with a 7% increase in fluorescence emission observed.

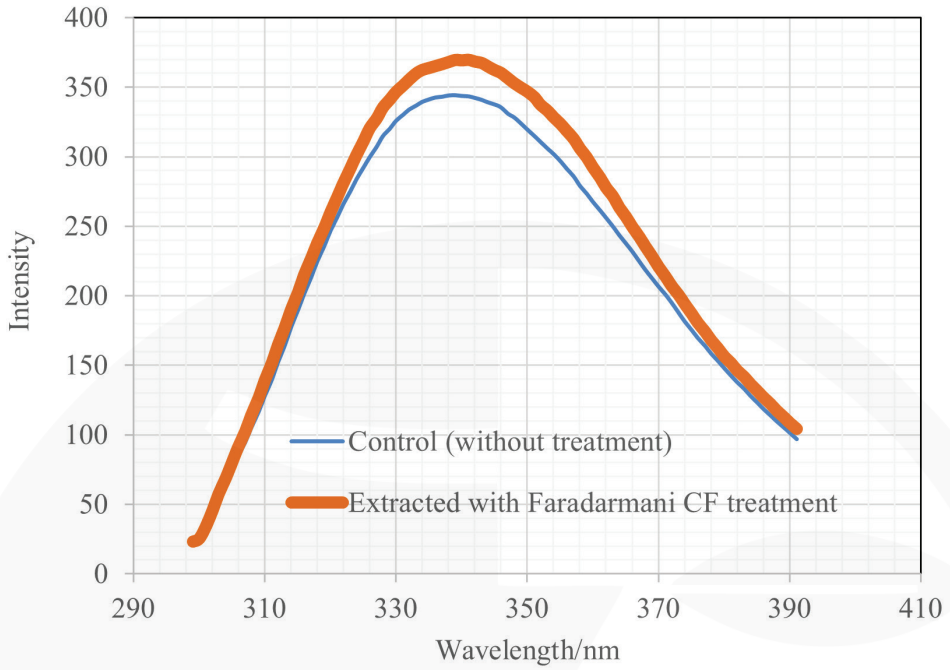


Figure 4. Fluorescence emission of plant-extracted HRP under the influence of the Faradarmani Consciousness Field compared to the control.

Discussion

In this study, the effect of the Faradarmani Consciousness Field on the structure and function of HRP was investigated in two forms: pure laboratory or commercial HRP and plant-extracted HRP. The observed changes, given that all conditions were identical between the samples exposed to the field and the controls (without field), provide evidence of the effect of this non-frequency Consciousness Field. In the commercial HRP sample, Faradarmani treatment led to an increase in Km, indicating a reduced binding affinity between the enzyme and its substrate (Bar-Even et al., 2011). In other words, a greater amount of substrate is required to reach half of Vmax. This observation is consistent with the decrease in fluorescence intensity. It is possible that changes occurred in the microenvironment or the structure of the active site, leading to a reduction in catalytic rate. Since no shift in the fluorescence peak was observed under the influence of the Faradarmani Consciousness field, this indicates that the field did not cause significant changes in the protein structure; in other words, denaturation did not occur. It is noteworthy that, in the case of the plant-extracted enzyme, the Faradarmani Consciousness Field, unlike the commercial sample, led to a 7% increase in fluorescence emission. This difference suggests that the plant-extracted enzyme, which has a different microenvironment, such as around the tryptophan residues (Gui et al., 2006), is affected by the Faradarmani differently compared to the commercial enzyme with higher purity.

Evolutionary studies have reported that over long-time scales, enzymes exhibit coordinated changes in Km and Kcat, reflecting modified metabolic roles (Pettersson, 1989). In contrast, kinetic constants measured under identical laboratory conditions usually vary only within the range of experimental error. The relatively large change observed here—particularly the increase in Km—suggests that the Faradarmani Consciousness Field may affect the functional or structural state of HRP. This increase in Km for the commercial enzyme was similarly observed in the plant-extracted enzyme when the

field was applied during the assay stage or during both extraction and kinetic assay, indicating the reproducibility of the field's effect.

As mentioned in the introduction section, the effects of T-Consciousness Fields are initiated without physical intervention and through the mind of the announcer. According to Taheri's theory, consciousness is a fundamental element of the universe, and not only beings with a brain, but every particle possesses a level of consciousness. This does not imply self-awareness or cognitive traits in advanced organisms such as humans. To better understand, the physical structure can be compared to computer hardware, which requires specific software and information to function. Although this software component is invisible, it plays a fundamental role in the computer's outputs and performance. Conventional science, and biology in particular, focuses on functions at the hardware level (matter and energy). Taheri's theory proposes that, in addition to matter and energy, there exists a third component called consciousness, which has no frequency or physical nature but influences their function. In this study, by examining the effects of the Faradarmani Consciousness Field, we provided evidence of its influence on enzymatic activity and structure. To better understand the mechanisms and mode of action of consciousness fields, further studies in biological, physical, and chemical contexts are needed.

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