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Beneficial effects of a Fermented Papaya Preparation for the treatment of electrohypersensitivity self-reporting patients: results of a phase I-II clinical trial with special reference to cerebral pulsation measurement and oxidative stress analysis

Irigaray Philippe[1], Garrel Catherine[2], Houssay Carine[1], Mantello Pierre[3], Belpomme Dominique [1,4*]

[1]European Cancer and Environment Research Institute (ECERI), Brussels, Belgium; [2]Pathology and biology institute, Centre Hospitalier Universitaire, Grenoble, France; [3]Osato Research Institute, Gifu, Japan; [4]Paris V University Hospital, France

Corresponding Author: Belpomme Dominique, European Cancer and Environment Research Institute (ECERI), Brussels, Belgium

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ABSTRACT

Background: Electromagnetic Field Intolerance Syndrome (EMFIS), also termed Idiopathic Environmental Intolerance (IEI) attributed to Electromagnetic Fields (IEI-EMF) by WHO, is a newly identified pathological disorder occurring in electrohypersensitivity (EHS) self-reporting patients. To date, there has been no recognized treatment of this disorder. We have shown that EHS self-reporting patients experience some degree of oxidative stress, inflammation, and autoimmune response. Additionally, Fermented Papaya Preparation (FPP) has some antioxidant, anti-inflammation, and immuno-modulating properties. The objective of this phase I-II clinical trial was thus to test whether FPP treatment is well tolerated, can improve clinical outcomes, and can normalize biological abnormalities.

Methods: 32 EMFIS-bearing patients were serially included in this trial, among which 26 and 16 of them were evaluable after 3 and 6 months of FPP treatment, respectively. Clinical assessment was conducted during a specific face to face interview by using a validated pre-established questionnaire. Biological assessment consisted of measuring intracerebral tissue pulsometric index (PI) in the temporal lobes with ultrasonic cerebral tomosphygmography (UCTS), in addition to oxidative stress and inflammation with a battery of oxidative stress and inflammation-related peripheral blood tests.

Results: Overall, clinical improvement was obtained in 50-60% of the cases, among which 20-35% presented major improvement that mainly consisted of the regression of cognitive symptoms such as loss of short term memory, concentration, attention deficiencies, insomnia, and fatigue. This clinical improvement was objectively supported by a statistically significant normal recovery of mean PI in the temporal lobes and by a FPP-related antioxidative effect, evidenced by a statistically significant decrease in malondialdehyde levels in the plasma ($p<0.0001$) and increase in the Glutathione peroxidase activity in red blood cells ($p<0.01$) in patients experiencing oxidative stress. Moreover, this trial evidenced some degree of FPP-related anti-inflammatory effects by demonstrating a statistically significant decrease in histamine ($p=0.049$) and HSP27/HSP70 chaperone proteins ($p=0.007$) in the peripheral blood of patients with initial increased values of these inflammation-related biomarkers.

Conclusion: The results suggest a beneficial clinical and biological therapeutic effect of FPP in EHS self-reporting patients. However, the precise underlying mechanism has not yet been elucidated.

BACKGROUND

Electromagnetic Field Intolerance Syndrome (EMFIS) is a new, emerging clinical disorder that has also been termed "idiopathic environmental intolerance (IEI) attributed to electromagnetic fields" (IEI-EMF) by the World Health Organization (WHO) [1]. This new pathological disorder, which occurs in so called electrohypersensitivity (EHS) self-reporting patients, has been shown to be associated with clinical symptoms such as headache, tinnitus, hyperacusis, superficial and/or deep sensibility abnormalities, skin lesions, fibromyalgia, vegetative system dysfunction, and reduced cognitive capability. All symptoms reported by these patients occur each time they are exposed to EMFs fields, which may result in chronic insomnia, fatigue, irritability, and depressive tendencies [2-6].

Recently, we clinically identified and biologically characterized this new disorder by showing that it is associated with a decrease in intracerebral tissue pulsations in the temporal lobes. In addition, there were several biological abnormalities in the peripheral blood, reflecting varying degrees of oxidative stress, inflammation, and autoimmune response. This led us to hypothesize that these processes may account for blood brain barrier (BBB) opening and decrease in brain blood flow (BBF) in the temporal lobes of these patients [7].

Fermented Papaya Preparation (FPP) is a recent biotechnology product resulting from yeast fermentation of the non-genetically modified medicinal plant *Carica Papaya Linn*, which was initially developed by Osato Research Institute in Japan and is currently marketed as a 100% natural dietary functional health supplement under the brand name Immun'Âge® in Europe, Asia, and the United States [8].

FPP has been shown to possess some anti-oxidant, anti-inflammatory, and immune-modulating properties in several pathological conditions [8-11], including neurodegenerative disorders [12]. This prompted us to test FPP in EMFIS-bearing patients, (i.e. in so called EHS self-reporting patients, in the framework of a prospective phase I-II clinical trial to demonstrate whether FPP is well tolerated, can improve clinical outcome, and can normalize biological abnormalities). In this trial, we paid special attention to the measurement of intracerebral tissue pulsometric index (PI) in the temporal lobes by using ultrasonic cerebral tomosphygmography

(UCTS) before and after FPP treatment. Additionally, we analyzed the FPP effects on oxidative stress and inflammation by measuring a series of biomarkers in the peripheral blood of these patients.

MATERIALS AND METHODS

Inclusion Criteria

In this study, we clinically defined EMFIS on the basis of five criteria: (1) Absence of known pathology accounting for the observed clinical symptoms; (2) Reproducibility of symptoms under the influence of EMFs, regardless of the incriminated source (radio-frequencies and/or hyper frequencies or low and/or extremely low frequencies); (3) Regression or disappearance of clinical symptoms in the case of EMF avoidance; (4) Clinical symptoms compatible with those previously reported in EHS self-reporting patients in the scientific literature; and (5) Chronic evolution of symptoms [4-7].

Therefore, before inclusion in this study, all patients had a general and neurological clinical examination and a systematic general biological check-up to exclude any EMFIS-non related pathology. Thus, to be included in the trial, patients should have a normal carotidian and vertebral echodoppler, a normal magnetic resonance imaging (MRI) or computed tomography (CT) scan before inclusion, and normal blood tests that are currently used, in particular hematologic, hepatic, and renal tests. Moreover, patients should be between 18 and 75 years old, have a body mass index between 18.5 and 25, normal peripheral blood pressure, normal fasting glycemia, no gluten and/or lactose/casein intolerance, and no multiple chemical sensitivity (MCS)-associated pathology.

Additionally, all patients should have no history of pathologies such as cancer, Alzheimer disease, type II diabetes, and/or cardiovascular disease, and should be in a clinical active phase of EMFIS, (i.e. with grade 2 or 3 clinical symptoms according to the grading scale system we used - see the section “evaluation”) whether they have been treated or not treated previously.

However, since clinical symptoms in so called EHS patients are mainly subjectively-expressed, we used two additional biological inclusion criteria to characterize objectively EMFIS bearing patients: (1) a mean low tissue PI measured in areas of at least one temporal lobe by using UCTS (since as we have previously reported, in EHS self-reporting patients there is a low mean tissue PI in several areas of temporal lobes [7]) and (2) increase of at least one of three peripheral blood biomarkers we have previously identified as being possibly associated with EHS [7]: Increased histamine, a mediator of inflammation [13]; increased protein S100B, a marker of oxidative stress-related BBB opening [14-15]; and increased chaperone proteins Hsp27 and/or Hsp70, markers of heat-shock cell stress-associated inflammation and/or immune response [16-17] (see Table 1).

This study was agreed to by the ECERI Scientific/Ethical advisory committee and was conducted according to currently accepted ethical guidelines, including informed written consent approval which was signed by all patients prior to the study. This non-invasive investigation has been also registered in the European Clinical *Trials* Database (*EudraCT*) under the registration number 2017-003937-27.

FPP Treatment and Study design

FPP was supplied by Osato research institute as sachets containing 4.5 grams of powder, one sachet being administered twice a day, morning and evening, according to the currently recommended use of this marketed product. In this study, FPP was administered during a 6-month period. The FPP components have been previously reported [8] (see discussion).

The study was an open prospective case-crossover phase I-II trial, each patient serving as its own control over time. A minimum of one-month washout period between the inclusion time (Ti) and therapeutic protocol initiation (T0) was used in the case of previously treated patients in order to avoid any bias due to an eventual backward effects of pre-inclusion treatments.

Additionally, patients were asked to maintain their usual lifestyle in order to avoid any confounding factors which may have modified their clinical symptoms during the study period. Their ordinary diet, physical activity, regular living behavior and working activity were systematically recorded and carefully analyzed during the study quality assurance process. Accordingly, any eventual modification should have been negligible for each included case to be considered evaluable.

Evaluation

Patients were serially investigated clinically and biologically at Ti and T0 and after the 3-month (T3) and 6-month treatment (T6). Clinical assessment was conducted systematically at T3 and T6 in comparison with T0 during a specific face to face interview by using a validated pre-established questionnaire and a complete clinical examination. The evaluation included FPP tolerance and clinical effects on EMFIS symptoms. Clinical assessment was performed using a symptomatic grading scale system: Grade 0 -no symptoms; Grade 1 - mild and/or transitory symptoms; Grade 2 - intensive or permanent symptoms; and Grade 3 - intensive and permanent symptoms. A shift from Grade 2-3 to Grade 0 was considered a "major" symptomatic improvement, while a shift from Grade 3 to Grade 2 or from Grade 2 to Grade 1 was categorized as a "minor" improvement. Overall clinical response at T3 or T6 was assessed in comparison with T0 by using the following grading system: "Complete response" (CR): disappearance of all symptoms (i.e. Grade 0); "Partial response" (PR): persistence of Grade 1 symptoms; "Stable" (S): no change; and "Failure" (F): increase of Grade 2 or 3 symptoms.

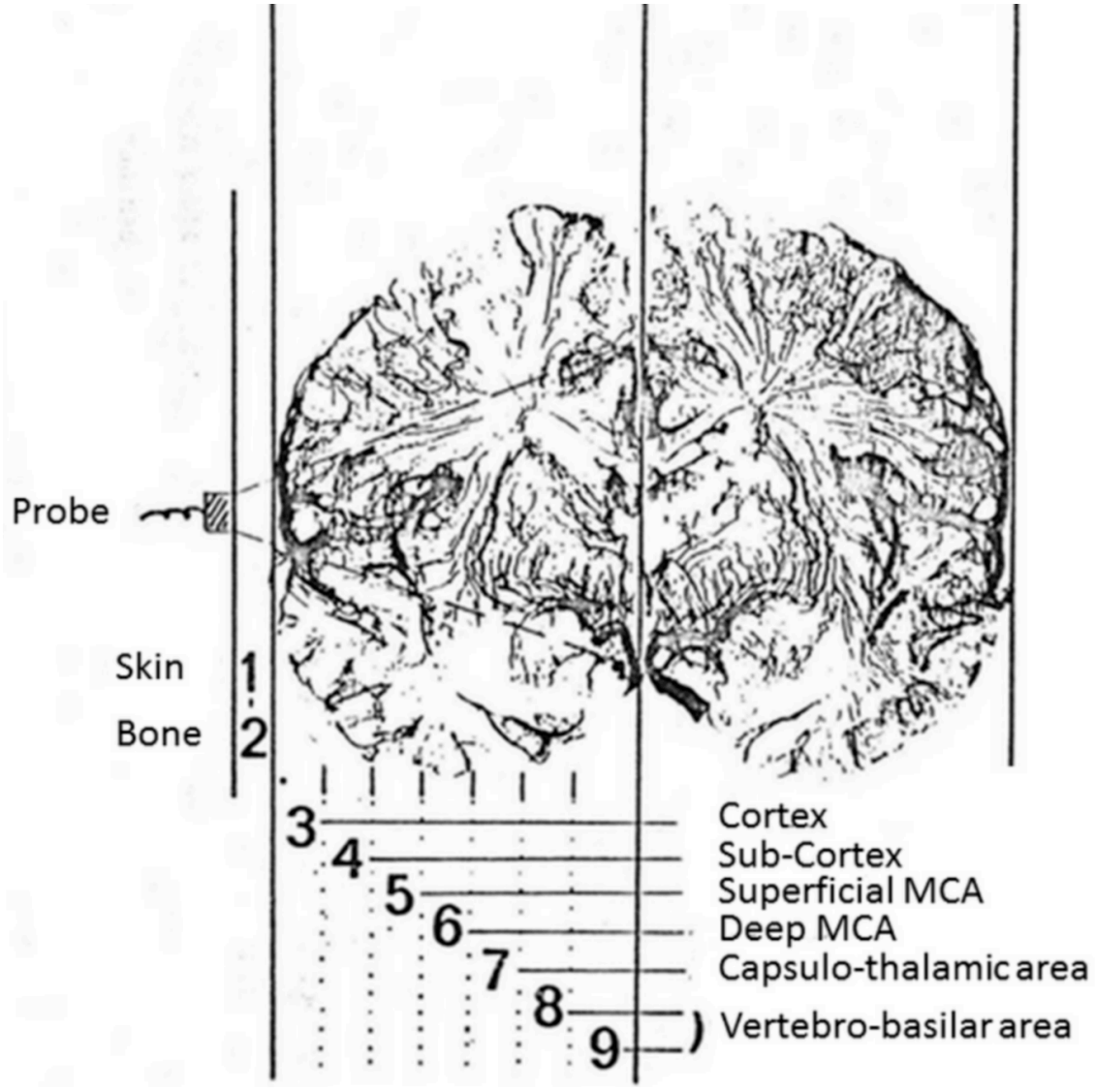
A complete biological evaluation was conducted at T3 and/or T6 in comparison with Ti or T0. This included a new PI measurement at T3 or T6 by UCTS for comparison with that of Ti; at T3 and T6 a new dosage of EMFIS-associated inflammation-related biomarkers in the peripheral blood (i.e. histamine, protein S100B, chaperone proteins Hsp27 and Hsp70) for comparison with Ti; and at T3 and T6 a new dosage of oxidative stress-related biomarkers for comparison with T0.

Ultrasonic cerebral tomosphygmography

After intracranial pulsation measurements were pioneered in the USA [18], UCTS was standardized and developed in France [19-20]. In this study, we rehabilitated this former intracerebral tissue-related ultrasonic technique to measure precise PI in the two temporal lobes because our preliminary clinical data have revealed that patients complaining with EHS most often have cognitive defects such as loss of short term memory, attention, and concentration deficiencies, in addition to frequent auditory and olfactory abnormalities. The UCTS technique

has been documented in our previous article [7]. UCTS consists of a non-invasive computerized technique that enables measurement of mean tissue PI by centimeter-thick sections of the temporal lobes from the cortex to the middle line of the brain through using the emission of 2 MHz pulsed ultrasonic waves at different intensity levels from a transmitting ultrasonic source (see Fig 1), in addition to the analysis of the reflection of these ultrasonic waves on the different neurologic and vascular tissue structures in the temporal lobe, particularly the red blood cells (RBC) in capillaries of the vascular network of the middle cerebral artery (MCA) [20].

Fig 1. Schematic diagram showing the different MCA-dependent areas individualized in the temporal lobes for the measurement of mean PI by using UCTS according to Parini et al. [20].



The addition of the mean PI value obtained at the 3rd cm from the cutaneous cranial surface to the mean PI value obtained at the 4th cm corresponds to the cortical/sub-cortical area. The addition of the mean PI values obtained from the 3rd cm to the 5th cm corresponds to the superficial area of the MCA. The addition of the mean PI values obtained from the 5th cm to the 7th cm, to the deep area of MCA and the mean PI value obtained at the 7th cm, corresponds to the capsulo-thalamic area. The addition of values obtained from the 3rd cm to the 7th cm corresponds to the

MCA dependent carotidian area. Finally, the addition of values obtained from the 8th cm to the 9th cm corresponds to the vertebro-basilar area.

Mean PI values corresponding to the different temporal lobe territories investigated were directly recorded by the standard UCTS equipment we used. In fact, it has been previously shown that the mean physiological PI values determined in the six temporal lobe tissue territories have been individualized differ from each other (see Fig 1). Since these different tissue territories were shown to correspond to different brain structures they have been termed respectively from the temporal lobe cortex to the brain middle line: carotidian, cortical-subcortical, superficial sylvian (or superficial MCA), deep sylvian (or deep MCA), capsulo-thalamic, and vertebro-basilar according to the main neurologic or vascular cerebral structure they are associated with, the so called carotidian territory corresponding to all the measured areas except the vertebro-basilar area.

As a result, by using UCTS we were able to measure mean PI in each of the different tissue territories investigated in all patients. In our previous ground-breaking publication, mean PI values were determined in 727 EHS and/or MCS patients and compared to a series of normal historical controls that have been used for the determination of the physiological mean PI reference values in each of the six individualized temporal lobe territories [20]. This allowed us to demonstrate that in comparison to normal subjects, cerebral pulsatility in patients with self-reporting EHS decreased or was even completely suppressed in several tissue areas in one or the two temporal lobes, most often in the capsulo-thalamic area, suggesting that within these territories BBF may be decreased in EHS and/or MCS patients [7].

Therefore, in the present study we used UCTS at Ti, T3, and/or T6 to measure PI in the temporal lobes before and after FPP administration to examine whether FPP is able to restore normal PI.

Inflammation- and oxidative stress-related biomarkers

One of the objective of this study was to measure inflammation and oxidative stress-related biomarkers in the peripheral blood of EMFIS-bearing patients before and after FPP treatment to demonstrate possible FPP anti-inflammatory and anti-oxidative stress effects.

Blood Collection

Venous whole blood sampling was performed at Ti, T0, T3, and T6 in laboratory "Labo XV" in Paris. EHS-related biomarkers were measured in this laboratory on serum, with the exception of histamine, which was measured on plasma after a whole blood collection on lithium heparinate. For oxidative stress-related biomarkers, whole blood was also collected on lithium heparinate and frozen immediately at -80°C before samples were sent to Laboratory Equinox, an academic research laboratory specialized in oxidative stress measurement and analysis in the University Hospital of Grenoble (France).

Inflammation-related biomarkers

The methods of measurement of the inflammation-related biomarkers we used in this study have already been published [7]. Tests and methods are indicated in Table 1. For histamine we used an ELISA specific test (Histamine ELISA RE59221 from IBL International GmbH) [21] and for protein S100B a quantitative automated chemiluminescent immunoassays (Liason S100 from

DiaSorin Deutschland GmbH) [22]; for the chaperone protein Hsp27 we used a quantitative sandwich ELISA assay test (Assay Designs™ Hsp27 ELISA kit) [23], and for the chaperone protein Hsp70 an Enzyme Immunometric Assay test (Assay Designs™ Hsp70 High Sensitivity Enzyme Immunometric Assay kit) [24]. All tests were performed using commercially available reagents, each patient value being compared to the normal reference value obtained from the commercial companies. Sensitivity, specificity, and reproducibility of the tests were in agreement with those provided by the companies. All assays were completed according to the recommended manufacturer's method.

Table 1. Investigated Inflammation-related biomarkers, and oxidative and antioxidative stress-related biomarkers.

	Biomarkers	**mple**	**Method used**
Inflammation-related biomarkers	Histamine	plasma	[21]
	Protein S100B	serum	[22]
	Protein Hsp27	serum	[23]
	Protein Hsp70	serum	[24]
Oxidative stress biomarkers	MDA	plasma	[25]
	TBARS	plasma	[25]
	Total thiol group	plasma	[26]
Antioxidative non-enzymatic proteins	GSSG	plasma	[27]
	GSH	plasma	[27]
	Total glutathione	plasma	[27]
	TAS	plasma	[28]
Antioxidative enzymes	SOD	RBC	[29]
	GR	plasma	[30]
	GR	RBC	[30]
	GPx	plasma	[31]
	GPx	RBC	[31]

MDA, Malondialdehyde; TBARs, Thiobarbituric acid reactive substances; GSSG, oxidized glutathione; GSH, Reduced Glutathione; TAS, Total antioxidant status; SOD, Superoxide dismutase; GR, Glutathione Reductase; GPx, Glutathione Peroxidase; RBC, red blood cells.

Oxidative and antioxidative stress-related biomarkers

We used a battery of tests to measure oxidative stress biomarkers and anti-oxidative non-enzymatic and enzymatic proteins in plasma and/or RBC before and after FPP treatment (Table 1). Measurements were completed after centrifugation (4000 g, 10 min, 4°C) to separate RBC from plasma. Normal reference values obtained for each biomarker we used in this study were pre-determined from the analysis of a series of 123 normal subjects.

Oxidative stress biomarkers

For oxidative stress assessment we measured the three following markers in the plasma: malondialdehyde (MDA), all thiobarbituric acid (TBA), reactive substances (TBARS), and total thiol group proteins.

For measuring MDA, we used its reaction with TBA. The MDA-TBA complex was then separated from interfering substances and specifically identified by using reverse-phase HPLC coupled with a UV/visible detection. In fact, MDA was quantified on the basis of its strong light-absorbing and fluorescing property following its reaction with TBA. Results were expressed in μmoles per liter (μM) [25]. For the dosage of lipid peroxidation intermediates we measured all plasma TBARS such as MDA by using a modification of the method of Ohkawa et al. [25]. This method is based on the reaction of the aldehyde function of TBARS with TBA to form a TBARS-TBA colored complex which was quantified by fluorometry. Results were expressed in μM. For the dosage of total thiol (SH) group proteins, we used 5, 5'- dithio-bis(2-nitrobenzoic acid) (DTNB) as reagent and measured the level of plasmatic SH group spectrophotometrically at 412 nm. Results were expressed in Unit per liter (U/l) [26].

Antioxidative non-enzymatic proteins

The dosage of reduced glutathione (GSH) and oxidized glutathione (GSSG) in the plasma was done according to the method of Akerboom and Sies [27]. Briefly, before centrifugation (400g, 10 min, 4°C), 400 μl of whole blood were collected in 3.6 ml of metaphoric acid. After centrifugation, total glutathione and GSH were measured enzymatically in the acidic protein-free-supernatant. The assay of GSSG was performed after having masked GSH by adding 2-vinylpyridine to the deproteinized extract. As for the total glutathione and GSH GSSG was measured enzymatically. Results were expressed in μM.

Finally, measurement of the total antioxidant status (TAS) in the plasma was done by a colorimetric method using a randox kit (Randox Total Antioxidant Status, NX2332, Randox laboratory). Results were expressed in μM [28].

Antioxidative enzymatic proteins

Measurement of antioxidative enzymes was done in RBC or both in RBC and plasma. For measuring Cu-Zn superoxide dismutase (SOD) activity in RBC we used the method described by Marklund and Marklund [29], which consists of a simple and rapid test based on the ability of SOD to inhibit the autoxidation of pyrogallol. Indeed, at pH 7.9 the reaction is inhibited by SOD, indicating the almost total effect of SOD on the superoxide anion radical, $0_2^{\circ-}$, in the reaction. In this method, the rate of pyrogallol auto-oxidation was determined spectrophotometrically from the increase in absorbance at 420 nm; one unit of SOD activity being defined as the amount of the enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%. Results were expressed in Unit/mg hemoglobin (U/mg Hb). For the dosage of Glutathione reductase (GR), we used a colorimetric method from a randox kit (GR2368 from Randox laboratory). Results were expressed in Unit/gram of hemoglobin (U/g Hb) for RBC GR, and Unit per liter (U/l) for plasmatic GR [30]. Additionally, Glutathione peroxidase (GPx) activity was measured in RBC and plasma using a method derived from Gunzler et al. [31]. The GPx assay was based on the

oxidation of NADPH to NADP+, which is accompanied by a decrease in absorbance at 340nm. The rate of this decrease is directly proportional to the GPx activity in the sample. GPx activity was then evaluated in nmoles per liter (nM) of NADPH oxidized per min and the results were expressed in Unit/gram of hemoglobin (U/g Hb) for RBC GPx and in Unit per liter (U/l) for plasmatic GPx.

Statistical analysis

The design of this study was a case-crossover clinical trial, which consisted of a within group comparison between the data obtained before and after the treatment.

Clinical assessment before and after FPP treatment was described as the following. Major and minor symptomatic improvements in addition to the overall response rates were established according to the clinical criteria previously defined in section "evaluation."

We used three different statistical tests: (1) the Chi-squared test of independence for comparison between the percentages of patients for the symptomatic (Tables 2 and 3) and biomarker assessment (Table 6); (2) the Fisher exact test followed by the two-tailed Student's t-test for comparison between the values obtained from two groups under comparison, such as the mean PI values relative to the mean normal control values (Table 5), or between the values obtained for the different oxidative stress-related biological parameters obtained from the patients with or without oxidative stress (Table 8); and (3) the Wilcoxon signed-rank test, as we hypothesized our data were not in agreement with a normal distribution for a within group comparison between the different values obtained at time measurement, such as for PI (Table 5) or biomarker analysis (Tables 7 and 9).

All statistical analysis was conducted using the XLSTAT software.

RESULTS

Of the 32 patients that were included, 26 were evaluable both for clinical symptoms and biological tests at T3. 5 cases were lost in the follow-up period between Ti and T3 and 1 case was not evaluable. At T6 18 patients were evaluable, 4 cases being excluded from the protocol at T3 due to failure and 4 other cases being lost in the follow-up period between T3 and T6.

Clinical assessment

In this study, all evaluable patients tolerated FPP well. Tables 2, 3, and 4 summarize our clinical data.

Major symptomatic improvement at T3 was obtained in 20-40% of the cases for loss of short term memory, impaired concentration/attention, insomnia, and fatigue. In contrast, there was improvement in only 5-15% of cases for depressive tendencies and for neurologic symptoms, such as headache, tinnitus, and dysesthesia. However, if we include the minor improvement, the total symptomatic improvement reaches about 30-60% of the cases, depending on the type of symptoms analyzed, which persists at T6 for those patients who reached 6 month FPP treatment (Table 2).

Table 2. Symptomatic evaluation at T3 and T6 in comparison with T0

	T3 n=26			**T6 n=18**
Symptoms	**Major improvement**	**Minor improvement**	**Total improvement**	**Total improvement**
Headache	2/26 (7.7 %)	12/26 (46.2 %)	14/26 (53.9 %)	8/18 (44.4 %)
Fatigue	11/26 (42.3 %)	7/26 (26.9 %)	18/26 (69.2 %)	14/18 (77.8 %)
Impaired concentration	10/26 (38.5 %)	6/26 (23.1 %)	16/26 (61.5 %)	14/18 (77.8 %)
Loss of short term memory	5/26 (19.2 %)	9/26 (34.6 %)	14/26 (53.8 %)	10/18 (55.6 %)
Attention deficit	7/26 (26.9 %)	6/26 (23.1 %)	13/26 (50 %)	11/18 (61.1 %)
Tinnitus	2/26 (7.7 %)	7/26 (26.9 %)	9/26 (34.6 %)	7/18 (38.9 %)
Insomnia	5/26 (19.2 %)	7/26 (26.9 %)	12/26 (46.2 %)	7/18 (38.9 %)
Depression tendency	3/26 (11.5 %)	7/26 (26.9 %)	10/26 (38.5 %)	14/18 (77.8 %)
Dysesthesia	2/26 (7.7 %)	8/26 (30.8 %)	10/26 (38.5 %)	11/18 (61.1 %)

This was confirmed in Table 3, which shows the percentages of patients with symptoms at T3 and T6 in comparison with T0 are statistically significantly decreased for each symptom analyzed ($p < 0.0001$).

Table 3. Evolution of the percentage of symptomatic patients at T3 and T6 in comparison with T0 for each symptom analyzed.

	Percent of patients with symptoms			**P-value***	
Symptoms	**T0**	**T3**	**T6**	**T3/T0**	**T6/T0**
Headache	90.63	46.88	65.63	< 0.0001	< 0.0001
Fatigue	90.63	34.38	46.88	< 0.0001	< 0.0001
Impaired concentration	81.30	31.30	37.50	< 0.0001	< 0.0001
Loss of short term memory	84.38	40.63	53.13	< 0.0001	< 0.0001
Attention deficit	81.25	40.63	46.88	< 0.0001	< 0.0001
Tinnitus	65.63	37.50	43.75	< 0.0001	< 0.0001
Insomnia	59.38	21.88	37.50	< 0.0001	< 0.0001
Depression tendency	56.25	25.00	12.50	< 0.0001	< 0.0001
Dysesthesia	53.13	28.13	9.38	< 0.0001	< 0.0001

**p* values relative to T0 values were calculated using the Chi-square test of independence.

Finally, a complete or partial clinical response was obtained at T3 in 14 of the 26 evaluable cases which resulted in an overall response rate of 53.8% (Table 4).

Table 4. Overall clinical response rates after 3 month FPP treatment (T3) in the 26 evaluable patients.

Response	**Number of patients**	
Non-evaluable	6/32 (18.8 %)	
Evaluable	26/32 (81.2 %)	
Complete response	3/26 (11.5 %)	14/26 (53.8 %)
Partial response	11/26 (42.3 %)	
Stability	8/26 (30.8 %)	12/26 (46.2 %)
Failure	4/26 (15.4 %)	

UCTS assessment

As indicated in Table 5 and Fig 2, we confirmed that by using UCTS at Ti there was a statistically significant decrease in mean PI in the capsulo-thalamic area of both temporal lobes and in the deep MCA area and the vertebro-basilar area of the right and left temporal lobe respectively. On the other hand, there was a statistically significant increase in the cortical-subcortical and superficial MCA areas in both temporal lobes. At T3/T6, we find a statistically significant recovery of normal mean PI in comparison to Ti for the capsulo-thalamic area of both temporal lobes, and for the deep MCA area and the vertebro-basilar area of the right and left temporal lobe respectively. Accordingly, with the exception of the vertebro-basilar area of the right temporal lobe which shows no PI recovery, all tissue areas for which there was a decreased mean PI value at Ti recovered at T3/T6 a statistically significant normal mean PI value after FPP treatment. In contrast, for all the other explored temporal areas with a normal or increased mean tissue PI value at Ti, mean PI values at T3/T6 were statistically significantly increased.

Table 5. Mean PI values (+/-SD) measured in the different MCA dependent tissue territories in the two temporal lobes. Effect of FPP treatment at T3/T6 in comparison with Ti.

Temporal lobe	**Tissue sections analyzed**	**Normal values**	**Ti**	**T3/T6**	**D%*** **	**P-value**** **
Right	carotidian	13 +/-2	11.60 +/- 2.80	22.52 +/- 5.26	94.20	< 0.0001
	cortical-subcortial	1.5 +/- 0.5	4.89 +/- 2.31*	8.61 +/- 3.46	75.83	< 0.0005
	superficial MCA	5 +/- 1	6.88 +/- 2.70*	12.86 +/- 4.10	86.73	< 0.0001
	deep MCA	12 +/- 2	6.69 +/- 2.20 **	13.91 +/- 3.10	107.90	< 0.0001
	capsulo-thalamic	5 +/- 1	2.02 +/- 0.86 **	5.28 +/- 1.63	160.93	< 0.0001
	vertebro-basilar	9 +/- 1	8.87 +/- 1.59	9.15 +/- 2.37	3.25	0.2636
Left	vertebro-basilar	9 +/- 1	4.79 +/- 2.86**	9.70 +/- 2.79	102.61	<0.0001
	capsulo-thalamic	5 +/- 1	3.46 +/- 1.68**	4.77 +/- 2.03	37.78	0.0151
	deep MCA	12 +/- 2	10.12 +/- 2.67	13.42 +/- 3.40	32.70	0.0018
	superficial MCA	5 +/- 1	8.48 +/- 3.30*	13.16 +/- 3.03	55.22	< 0.0001
	cortical-subcortial	1.5 +/- 0.5	5.18 +/- 2.33*	9.21 +/- 2.25	77.74	< 0.0001
	carotidian	13+/-2	15.30 +/- 4.00	22.63 +/- 4.40	47.96	< 0.0001

SD, Standard deviation; Ti, Inclusion time; T3, UCTS assessment after 3 month treatment from the date of treatment initiation (T0); T6, UCTS assessment after 6 month treatment from T0.

*Mean PI values at Ti relative to normal reference values are statistically significantly *increased* using the Fisher exact test followed by the two-tailed Student's t-test ($p< 0.001$) for the superficial MCA and cortical-subcortical areas of the right and left temporal lobes.

** Mean PI values at Ti relative to normal reference value are statistically significantly *decreased* using the Fisher exact test followed by the two-tailed Student's t-test. The difference is statistically significant at $p<0.0001$ for the capsulo-thalamic area and the deep MCA area of the right temporal lobe and for the vertebro-basilar area of the left temporal lobe, while being $p<0.01$ for the capsulo-thalamic area of the left temporal lobe.

*** D% is calculated according to ((t3/t6 – ti) / ti) x 100, where t3/t6 and ti are mean PI values at T3/T6 and Ti. It corresponds to the percent mean value change between T3/T6 and Ti.

****p values for comparison between mean PI values obtained at T3/T6 and Ti were calculated using the Wilcoxon signed-rank test.

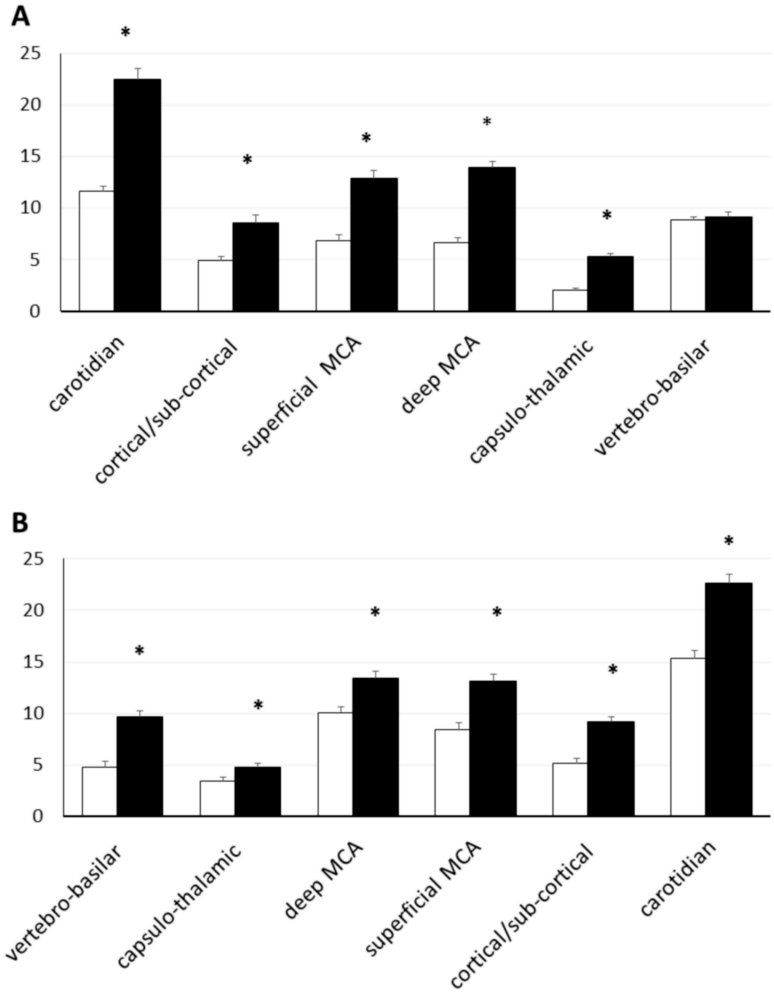


Fig 2: Mean PI values recorded by UCTS in the different territories of each temporal lobe investigated at T3/T6 in comparison with Ti.

(A) Right temporal lobe. (B) Left temporal lobe. White columns correspond to mean values +/- standard error (SE) at Ti and black columns to mean +/- SE at T3/T6.
*Statistically significant values using the Wilcoxon signed-rank test.

Inflammation-related biomarkers

Tables 6 and 7 depict the results we obtained at T3 and T6 in comparison with Ti for the inflammation-related biomarkers in EHS-bearing patients presenting with increase in these biomarkers at Ti.

As indicated in Table 6 there is a statistically significant decrease in the number (and percentage) of EHS patients with abnormal inflammation-related biomarker values at T3 and T6 in comparison with Ti ($p < 0.0001$) for histamine, S100B protein, and HSP27/70 chaperone proteins, suggesting the occurrence of some FPP-related anti-inflammatory effect in the peripheral blood of EHS patients with initially detectable inflammation. Moreover, as indicated in Table 7, the FPP-related anti-inflammatory effect was confirmed by comparing the mean+/-SD values of histamine and Hsp27/Hsp70 chaperone proteins at T3 and T6 with those at Ti, the decrease in mean values+/-SD being statistically significant at T3 and T6 relative to Ti.

Table 6: Number and percentage of EHS-bearing patients with increased inflammation-related biomarker values at T3 and T6 in comparison with Ti

	Normal values (Units)	Patients with increased values at Ti	Patients with increased values at T3	Patients with increased values at T6	P-value* T3/Ti	P-value* T6/Ti
Histamine	≤ 10 nmol/l	11 (42.3 %)	4 (15.4 %)	3 (16.7 %)	<0.0001	<0.0001
S100B protein	≤ 0.105 µg/l	6 (23.1 %)	3 (11.5 %)	0	0.03	<0.0001
Hsp27/Hsp70 proteins	≤ 5 ng/ml	17 (65.4 %)	5 (19.2 %)	2 (11.1 %)	<0.0001	<0.0001

*p values relative to Ti values were calculated using the Chi-square test of independence

Table 7. Mean values (+/- SD) of inflammation-related biomarkers at T3 and T6 in comparison with Ti in EHS-bearing patients having initial detectable increased inflammation-related biomarkers.

	Normal values (Units)	Ti	T3	T6	P-value* T3/Ti	P-value* T6/Ti
Histamine	≤ 10 nmol/l	23.22 +/- 9.79	12.03 +/- 11.90	8.73 +/- 8.06	0.049	0.006
S100B protein	≤ 0.105 µg/l	0.188 +/- 0.104	0.097 +/- 0.002	0.08 +/-0.02	0.142	0.098
Hsp27/Hsp70 proteins	≤ 5 ng/ml	9.02 +/- 7.41	4.29 +/- 1.76	3.34 +/- 1.98	0.007	< 0.001

*Comparison was done using the Wilcoxon signed-rank test.

Oxidative stress and antioxidative stress-related biomarkers

Results are depicted in Tables 8 and 9. A remarkable finding is that 12 of the 26 evaluable patients (46%) experienced oxidative stress. This was evidenced by the observation that at T0 these patients presented with statistically significant increase in MDA and TBARS in the plasma

relative to the normal reference values, while these values differed statistically significantly from those obtained in the patients with no detectable oxidative stress ($p<0.0001$). Moreover, the individualization of the group of patients with oxidative stress from that with no oxidative stress was further supported by the fact that in comparison with normal reference value, GSSG was statistically significantly increased in oxidative stress bearing patients ($p<0.0001$), but not in patients with no oxidative stress (Table 8).

Table 8. Individualization and characterization of a group of EHS self-reporting patients with oxidative stress at T0 and comparison between the two groups with or without oxidative stress with regards to the mean values +/- SD obtained for the different oxidative stress biomarker analyzed.

Oxidative stress biomarkers	**Normal reference values**	**Patients with oxidative stress (n=12)**	**Patients without oxidative stress (n=20)**	**P-value****
MDA	1.47 +/- 0.35 µM	2.14 +/- 0.17*	1.53 +/- 0.21	< 0.0001
TBARS	2.5 +/- 0.37 µM	3.16 +/- 0.18*	2.67 +/- 0.23	< 0.0001
Total thiol group	6.79 +/- 0.99 µmoles/g	6.56 +/- 0.46	6.51 +/- 0.44	0.74
GSSG	12.40 +/- 6.90 µM	24.08 +/- 12.66*	18.74 +/- 7.37	0.14
GSH	966 +/- 237 µM	848.38 +/- 160.18	822.12 +/- 149.47	0.64
Total glutathione	988.50 +/- 239.50 µM	896.58 +/- 175.40	859.60 +/- 148.79	0.53
GSH/Total glutathione ratio	97 +/- 2.90 %	94.75 +/- 1.99	95.54 +/- 1.75	0.25
GSH/GSSG ratio	97.55 +/- 57.45 µM/µM	41.20 +/- 15.75	50.35 +/- 23.02	0.23
TAS	1.50 +/- 0.15 µM	1.46 +/- 0.05	1.47 +/- 0.09	0.73
SOD	1.34 +/- 0.12 U/mg Hb	1.47 +/- 0.14	1.51 +/- 0.09	0.42
GR plasma	54 +/- 21 U/l	65.42 +/- 8.22	60.50 +/- 9.03	0.14
GR RBC	8.95 +/- 4.25 U/g Hb	9.83 +/- 1.28	9.18 +/- 2.23	0.36
GPx plasma	375 +/- 75 U/l	399.00 +/- 33.86	367.45 +/- 58.80	0.10
GPx RBC	44.15 +/- 16.35 U/g Hb	53.53 +/- 10.80	50.95 +/- 8.18	0.45

MDA, Malondialdehyde; TBARs, Thiobarbituric acid reactive substances; GSSG, oxidized glutathione; GSH, Reduced Glutathione; TAS, Total antioxidant status; SOD, Superoxyde dismutase; GR, Glutathione Reductase; GPx, Glutathione Peroxidase.

* Mean values +/- SD are statistically significantly higher than the normal reference values by using the Fisher exact test followed by the two-tailed Student's t-test ($p < 0.0001$).

**For comparison between patients with oxidative stress and without oxidative stress, p values are calculated by using the Fisher exact test followed by the two-tailed Student's t-test.

Table 9. FPP-related antioxidative response at T3 in comparison with T0 in EHS self-reporting patients with or without oxidative stress

	Patients with oxidative stress			**Patients without oxidative stress**		
Oxidative stress biomarkers	**T0**	**T3**	**P-value****	**T0**	**T3**	**P-value****
MDA	2.14 +/- 0.17*	1.82 +/-0.17	< 0.001	1.54 +/- 0.23	1.44 +/- 0.29	0.28
TBARS	3.16 +/- 0.18*	3.01 +/- 0.26*	0.13	2.65 +/- 0.24	2.58 +/- 0.33	0.47
Total thiol group	6.56 +/- 0.46	6.50 +/- 0.39	0.67	6.53 +/- 0.47	6.60 +/- 0.39	0.60
GSSG	24.08 +/- 12.66*	23.26 +/- 10.97 *	0.73	19.57 +/- 7.98	18.73 +/- 6.32	0.60
GSH	848.38 +/- 160.18	852.85 +/- 123.68	0.85	828.81 +/- 136.72	761.41 +/- 157.05	0.19
Total glutathione	896.58 +/- 175.40	899.33 +/- 142.76	0.97	867.87 +/- 135.80	798.87 +/- 158.15	0.15
GSH/Total glutathione ratio	94.75 +/- 1.99	95.02 +/- 1.62	0.58	95.43 +/- 1.82	95.15 +/- 1.97	0.60
GSH/GSSG ratio	41.20 +/- 15.75	42.01 +/- 13.69	0.85	49.12 +/- 23.48	45.70 +/- 18.84	0.72
TAS	1.46 +/- 0.05	1.46 +/- 0.08	0.76	1.45 +/- 0.07	1.46 +/- 0.11	1
SOD	1.47 +/- 0.14	1.51 +/- 0.14	0.22	1.49 +/- 0.08 *	1.52 +/- 0.09*	0.12
GR plasma	65.42 +/- 8.22	66.83 +/- 8.67	0.29	61.07 +/- 10.03	60.80 +/- 11.71	0.21
GR RBC	9.83 +/- 1.28	9.97 +/- 2.09	0.85	8.67 +/- 1.86	9.16 +/- 2.08	0.15
GPx plasma	399.00 +/- 33.86	378.70 +/-125.95	0.57	365.73 +/- 42.80	382.13 +/- 38.90	0.06
GPx RBC	53.53 +/- 10.80	56.22 +/- 11.05	< 0.01	52.46 +/- 7.80	48.37 +/- 17.58	0.45

MDA, Malondialdehyde; TBARs, Thiobarbituric acid reactive substances; GSSG, oxidized glutathione; GSH, Reduced Glutathione; TAS, Total antioxidant status; SOD, Superoxyde dismutase; GR, Glutathione Reductase; GPx, Glutathione Peroxidase.

* Mean values +/- SD relative to normal reference values are statistically significantly increased at TO for MDA, TBARs, and GSSG for the patients with oxidative stress and for SOD for the patients without oxidative stress by using the Fisher's exact test followed by the two-tailed Student's t-test ($p < 0.0001$).

***p* values were calculated using the Wilcoxon signed-rank test.

As indicated in Table 9, an additional observation is that for the group of patients with oxidative stress at T3 in comparison with T0 there is a statistically significant decrease of MDA level in the plasma ($p<0.0001$) and a statistically significant increase in the glutathione peroxidase activity in RBC ($p<0.01$). This result suggests that FPP may have some detectable antioxidative effect in patients with initially detectable oxidative stress. However, in this group of patients, the evidence for such an effect was not supported for the thiol group marker, whose normal level did not change at T3 and for TBARS and GSSG, since in comparison with the normal values the plasmatic values of these two markers were still statistically significantly increased at T3. In fact, regardless of the group of patients considered (i.e. with or without oxidative stress) we were unable to detect any statistically significant effect of FPP on the antioxidative enzymatic or non-enzymatic proteins so far investigated, with the exception of the increased glutathione peroxidase activity in RBC in the oxidative stress-related group.

DISCUSSION

To our knowledge, this is the first study to demonstrate some beneficial therapeutic effects in EHS self-reporting patients. In previous works, EHS or similar environmental pathological disorders such as MCS were categorized as toxicant-induced loss of tolerance (TILT) disease [32] or as sensitivity-related illness (SRI) [33], to account for the fact that EHS and MCS patients cannot tolerate weak EMF intensity and weak chemical concentration respectively. More recently we defined EHS more precisely as a decrease of EMF tolerance threshold and extension of this decreased threshold to the whole electromagnetic spectrum as disease progresses [7]. Since we were not able to measure precisely the EMF tolerance threshold in the patients included in this study and provide the supporting evidence that symptoms may have occurred in the case of weak EMF intensity, we preferred to use the more general term EMFIS to qualify the clinical and biological pathological condition these patients were associated with. Although WHO officially recognized EHS as an adverse health condition [34], a similar approach was proposed following the Prague WHO sponsored international workshop on EMF hypersensitivity, as it was recommended to use the term IEI-EMF [1] to qualify such pathology because there is still no proven causality between EMF exposure and EHS genesis.

In the present study, the clinical symptoms we analyzed correspond to those reported in the scientific literature for EHS self-reporting patients [2-6]. Currently, we have no clear explanation why after FPP treatment the cognitive symptoms including loss of short term memory and/or attention/concentration deficiencies were more frequently improved than the neurologic symptoms, which included headaches, tinnitus, or dysesthesia. From the analysis of Table 2, it appears that several symptoms such as fatigue, impaired concentration, depression tendencies, and dysesthesia may have improved at T6 in comparison with T3. Unfortunately, this is likely not the case because at T6, there were only 18 evaluable cases, 4 cases not being considered at T6 due to failure at T3 and 4 other cases because of the loss of follow-up and the undetermined outcome. As a result, such a selection process may have thereby artificially overestimated this apparently beneficial effect at T6. Due to this limitation, we cannot consider that FPP may have a permanent beneficial effect. Although we could not eliminate the possibility of some placebo effect to explain the general beneficial effect we observed, we believe such a placebo effect may have been relatively modest since the improvement has been observed not only at the standpoint of subjective clinical symptoms but also objectively through the use of UCTS and biomarker

detection. In order to clearly answer this important new clinical research question, a randomized trial testing the effect of FPP versus a placebo is necessary, a step which we are currently undertaking.

Since UCTS measure tissue pulsations of centimeter-thick sections corresponding to intracerebral tissue territories vascularized by MCA but not pulsation of the MCA itself, this technique must be distinguished from the classical transcranial Doppler ultrasonography (TDU), which measures cerebral perfusion pressure upon MCA blood flow velocity [35-36].

It is well known that using transcranial ultrasound techniques such as TDU or UCTS factors that affect cerebral pulsations can be extracranial or intracranial [18]. Among the extracranial factors there are peripheral vascular changes, such as low systolic arterial pressure. However, in this study, at Ti all patients had a normal systemic arterial pressure and a normal carotidian and vertebral echodoppler. This led us to consider that intracranial factors such as local vascular changes and/or changes in brain tissue metabolism and function may be involved to account for the low mean PI detected by UCTS in the different temporal lobe areas investigated.

In an ongoing study, by using TDU in association with UCTS we found some decrease in MCA blood flow velocity in the brain of EHS self-reporting patients for whom we had simultaneously evidenced a decrease in intracerebral tissue PI in temporal lobes, suggesting the decreased PI measured by UCTS may be associated in these patients with a decrease in MCA-related BBF [7]. This finding may confirm other publications which have shown by using other imaging techniques that the excessive use of mobile phones (i.e. the prolonged exposure to pulse-modulated radio frequency EMF) can affect regional BBF [37-38]; and that BBF disruption may consequently disturb sleep and waking EEG [39]. Moreover, it has been clearly established experimentally that 900 MHz or 2.45 GHz microwave short term or chronic EMF exposure in rats can trigger neuronal dysfunction and even apoptosis of hippocampal pyramidal cells [40-42] and cerebellum Purkinje cells [43-44] through oxidative stress induction, and that EMF-related oxidative stress-induced neuronal pathologic changes may transmit to offspring [44].

The statistically significant decrease in mean PI values evidenced at Ti in the MCA dependent temporal lobe territories investigated by using UCTS may thus similarly be associated with brain tissue metabolic changes in the limbic system and the nearby temporal lobe neuronal structures. Such pathological changes may indeed be related to oxidative stress-induced BBB opening [45], and/or to brain hypoxia caused by EMF-induced BBF decrease and/or EMF-induced hemoglobin deoxygenation [46-47]. Consequently, this may induce metabolic neuronal dysfunction but not apoptosis. For example, in our study, with the exception of the vertebro-basilar area of the right temporal lobe, a complete mean PI recovery was observed as soon as 3/6 month FPP treatment was achieved. This is particularly true for the capsulo-thalamic area which was constantly associated with a decrease in mean tissue PI in one or the two temporal lobes at Ti and which comprises the limbic system (i.e. the hippocampus and the amygdale) and the thalamus. We hypothesize this area is particularly critical since it may contain the multisensory parieto-temporal cortex zone which appears to be connected with the thalamus for integration of auditory and somatosensory responses, as it was demonstrated in the rat [48]. Regardless of the mechanisms, dysfunction of the limbic system and the thalamus may account for the cognitive deficiency and the superficial and/or deep sensibility abnormalities we observed clinically before FPP treatment. We have also previously reported that similar PI decrease in the capsulo-thalamic

area can be evidenced in MCS patients [7]. Since we have shown that MCS is associated with EHS in many patients, it cannot be excluded that chemicals may also be involved as causal environmental stressors in EHS genesis.

In the present study, all the included patients for whom a MRI or CT scan could have been conducted before inclusion had a normal brain MRI or CT scan. Accordingly, abnormalities in the limbic system and/or in the thalamus could not be detected using these typical imaging techniques. However, by using more sophisticated imaging techniques such as positron emission tomography (PET) or functional MRI (fMRI), it has been possible to observe some metabolic hyperactivity in the limbic amygdale of MCS patients (PET studies) [49] and some abnormal default mode network, including decreased BBF and/or metabolic activity within bi-frontal lobes in the brain of EHS patients (fMRI studies) [50].

In the present, we have no clear explanation how FPP can restore normal mean PI values and how it can increase mean tissue PI values in comparison with normal values at T3/T6 in the superficial MCA, cortical-subcortical and carotidian areas of the left and right temporal lobes (see Table 5). UCTS related-mean PI decrease in temporal lobes has been shown to be associated with brain dysfunction [19-20]. There is no data which demonstrates that increased mean tissue PI is pathologic. In fact, in our study the FPP-related increase in mean tissue PI in these temporal lobe areas was transitory, as several months after FPP treatment concluded mean PI values were normal or even decreased.

Analysis of the general composition of FPP has revealed it contains 91.3% of carbohydrates, <0.1% of lipids, and only 0.3% of proteins for which all amino acids, mainly arginine, leucine, glutamic acid, and aspartic acid but not cysteine are represented [8]. The precise role of these plant-derived natural components on health is not known. However, FPP has been shown to produce some anti-inflammatory and anti-oxidant effects in chronic degenerative disease such as Alzheimer disease [12]. Similar favorable effects have also been observed for the treatment of diabetes [9] and for the prevention of psychological stress-induced acute gastric mucosal lesions [10], oxidative stress-induced oral cavity mucosal inflammation [11], and oxidative stress-associated occupational burn out [51].

On the basis of our previous data [7], we have speculated that BBF decrease and BBB opening may be caused by EMF and/or chemical-induced inflammatory and oxidative stress processes in the temporal lobes, thereby avoiding any psychosomatic or nocebo causal effects in EHS and/or MCS genesis. This hypothesis was supported by the numerous animal experiments which clearly demonstrate that EMFs can cause oxidative stress [52-53] more particularly in the brain [42, 54-56], and that in tested animals EMFs causally affect the test group but not the sham control group [40-42, 55, 57]. Our hypothesis has been recently confirmed in humans within a carefully-conducted interview-based psycho-clinical study showing EHS patient symptoms appear a long time before patients start questioning themselves on the EMF's impact on their health. An observation inconsistent with the hypothesis that EHS originates from a nocebo response to perceived EMF exposure [58].

We have examined whether FPP treatment could bring about some anti-inflammatory and antioxidant effects. To this end we have used a battery of biomarkers (see Table 1) chosen for their ability to reflect the general inflammation and redox state in the organism. An important observation in our study is that FPP can decrease the peripheral blood levels of histamine and Hsp27/Hsp70 chaperone proteins in patients where levels of these inflammation-related

biomarkers were initially elevated, a finding which confirms that FPP can bring about some anti-inflammatory effect. MDA and TBARS plasmatic values were found at T0 to be statistically significantly increased in comparison with normal reference values ($p<0.0001$) in 12 patients. This allowed us to individualize a group of patients with oxidative stress which represent 46% of the total number of evaluable patients (Table 8). MDA is one of the most prevalent by-products of lipid peroxidation during oxidative stress. This is also the case for TBARS, which include MDA, with both by-products being markers of lipid peroxidation [59]. A further finding which strengthens our proposed distinction between EHS patients with and without oxidative stress is that relative to the normal reference value GSSG levels were also found to be statistically significantly increased in the oxidative stress group ($p<0.0001$) but not in the group with no oxidative stress (see Table 8).

Regarding the anti-oxidative effect of FPP, we found a statistically significant decrease in MDA plasmatic levels and a statistically significant increase in glutathione peroxidase activity in RBC at T3 in the group of patients with oxidative stress (see Table 9), a finding which confirms that FPP may have also some systemic antioxidant properties.

In fact, papaya juice has been shown to scavenge free radicals [60] and it is believed that polyphenols in FPP may have such an antioxidant effect [11] by enhancing the total oxidant-scavenging capacities of human blood by binding to RBC [61]. Another antioxidant mechanism could be that FPP may act by inhibiting the Fenton reaction [62] by causing iron chelation [63]

CONCLUSION: As we observed some clinical improvement, an UCTS-based tissue PI normalization in temporal lobes, and some anti-inflammatory and antioxidative stress effects, we conclude that FPP is a useful treatment for EMFIS-bearing patients, i.e. for so called EHS self-reporting patients.

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Conflict of interest

At the exception of Dr. Pierre Mantello who is in charge with the marketing and communication of Immun'Âge®, all the authors declare no financial conflict of interest.

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