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Effects of dietary supplementation with fermented papaya on oxidative stress, symptoms, and microbiome in Parkinson's disease

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ABSTRACT

Background: Oxidative stress, understood as the alteration of the physiological equilibrium between the production of oxygen and nitrogen free radicals and their metabolic neutralization (redox imbalance), is a typical condition of several pathologies, including neurodegenerative ones.

In Parkinson's disease (PD), oxidative phenomena typically interest the dopaminergic neurons of mesencephalic substantia nigra. Although it is unlikely that the redox imbalance represents the primary event of neurodegeneration, it is certain that it participates in cellular damage progression.

Objectives: Interventions to prevent or reduce the extent of the oxidative stress in PD and the consequent oxidative damage are of crucial importance. With this study, we have evaluated the effects of prolonged treatment with fermented papaya preparation (FPP) on redox imbalance, clinical parameters, and intestinal microbiome of PD patients.

Methods: For six months, one group of PD subjects were treated with FPP (n=19, verum) and another with placebo (n=20, control); then, in the following six months, the treatments were exchanged.

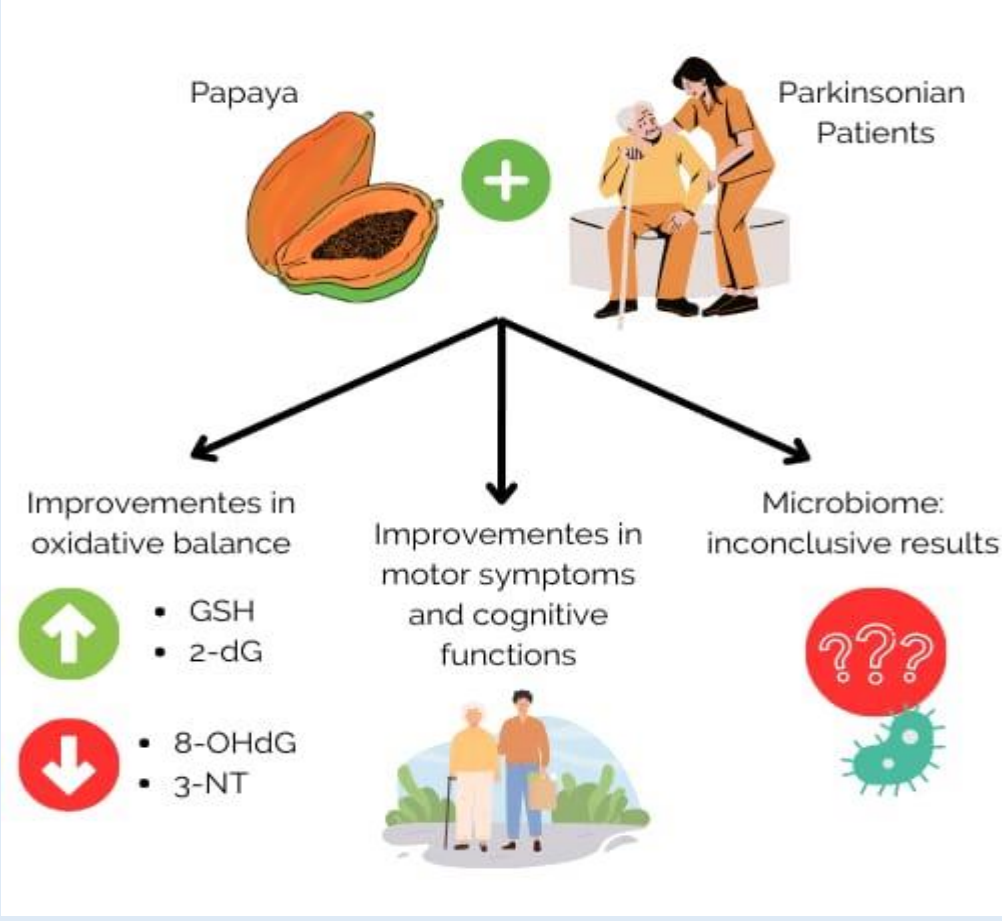
Several blood biochemical and hematological parameters were measured at the start and at the end of treatments. Among them are some components of antioxidant barriers, free radicals (total peroxides) and biomarkers of oxidative damage on DNA and proteins. To check the effects of FPP treatment on intestinal bacterial flora, we also evaluated the

modification of microbiome with regards to the relative amounts of different phyla, families, genera, and species. Furthermore, accurate evaluations were performed on motor symptoms and cognitive functions of patients with validated survey scales to check the effects of FPP treatment on clinical parameters and quality life.

Results: Unlike the control group, the level of free radicals in the patients treated with FPP was not increased; the antioxidant barrier was strengthened and oxidative damages on proteins and especially on DNA were decreased. Even clinical features and quality life parameters of these patients have improved. Instead, the results of microbiome were inconclusive as changes resulted seemingly independent Ofelia the treatments.

Conclusions: The study demonstrates that FPP may be a valuable aid in counteracting oxidative stress and improve the motor symptoms and cognitive functions in PD. This effect does not seem to depend on increased growth of a particular bacterial phylum because the microbioma composition does not change significantly following the treatment; it cannot be excluded, however, that FPP works otherwise by modifying not the quantity but rather the metabolism of some specific bacterial group or that it has effects on the integrity of the intestinal mucosa.

Keywords: Papaya, oxidative stress, Parkinson's disease, microbiome



INTRODUCTION

The cause of cell death in neurodegenerative diseases is still unknown. However, the overproduction of "free radicals," more properly, oxygen (ROS) and nitrogen reactive species (RNS), the consequent redox imbalance and the appearance of molecular alterations deriving from oxidative damages are frequent in these conditions. In PD, many alterations of cerebral substantia nigra have been described, including disfunctions of iron metabolism, mitochondrial activity and endogenous antioxidant defenses which together can lead to a pro-oxidant environment that evolve into oxidative stress.

The overproduction of free radicals may cause oxidative damage on lipids, proteins, and DNA bases [1-12] that do not seem however related to the chronic administration of levodopa [13].

Marked oxidative damage has been described in the basal ganglia as well as in other degenerative diseases, such as multiple systemic atrophy, progressive supranuclear palsy, and Huntington's disease [14-16]. Studies on Alzheimer's disease and diabetic neuropathy have similarly suggested the involvement of free radicals in neurodegeneration [17-20]. Therefore, it is suggestive to think that oxidative stress could represent a common pathogenetic mechanism.

Several studies on the effects of dietary supplementation with multivitamin complexes, amino acids and trace elements have reported conflicting conclusions. Some authors have demonstrated a substantial ineffectiveness of these treatments [21-22], some others pro-oxidant effects [23], and others, more recently, their success [24-25].

Although there is a wideness of conditions and parameters assessed in many studies, it makes it difficult to come to univocal conclusions. The current "state of art" suggests that the effectiveness of supplementation is greater when the treatment regards subjects who really demonstrate deficiency states. Lack of vitamins [26-27] and trace elements [28-30] have been repeatedly described in PD, probably as consequence of some of the so-called non-motor symptoms that often accompany the syndrome [31-32]. In fact, the nigro-striatal damage that causes the characteristic motor symptoms of pathology seems to be preceded by alterations of vagus, the main parasympathetic innervation of the gastrointestinal system [33].

This condition, together with the frequent dysphagia, constipation and the competitive interaction between food and levodopa, can negatively influence the nutritional status of PD patients, which can consider subjects at elevated risk of malnutrition. They would require nutritional rehabilitation and could benefit from appropriate integration and supplementation interventions.

Recent studies have also demonstrated the reoccurrence of leaky gut syndrome in PD, in which epithelial membrane integrity is lost and translocation of bacteria from the lumen to the mucosa triggers neuroinflammation of enteric glia and precipitation of alpha- misfolded synuclein. These conditions, together with the symptomatic gastrointestinal motility impairment, may be considered prodromal symptoms of PD and suggest the implication of the intestinal microbial flora in pathogenesis of the disease [34].

Some Authors have tried to focus the pathogenetic noxa with metabolomic analyses of large panels of low molecular weight substances, such as amino acids, organic and fatty acids in various cell types, tissues, organs, and biological fluids [35-39].

Reflecting the state of tissues, the metabolome can provide a picture of how the system responds to a specific alteration. Changes in metabolome have been

shown in PD related to epigenetic factors such as drug treatment, nutrient availability, genetic modification, mitochondrial dysfunction, and oxidative stress [36,40]. Because the studies on nutritional status [41] and on administration of antioxidants in combination with levodopa [42] demonstrated the efficacy of multinutrients and probiotic food supplementation in contrasting the mitochondrial decay, it is suggestive to think that similar interventions may constitute a strategy in prevention of PD and in slowing its course [43-44].

Fermented papaya (FPP) is a natural food rich in amino acids produced by bio-fermentation of the fruits of the *Carica papaya* which has been proven effective in modulating directly and indirectly the redox balance. It has been hypothesized that FPP acts both as a direct antioxidant, thanks to its molecular composition, and as a gene up-regulator of enzymes such as catalase, glutathione peroxidase, superoxide dismutase and other molecules involved in strengthening the antioxidant barrier and repair mechanisms of the oxidative damage [45-46].

In a previous study we had already demonstrated the biochemical improvements of the redox balance in PD patients after a FPP treatment [47], but with this new work, we wanted to check the hypothesis that these improvements depended on modifications induced by FPP on intestinal bacterial flora. For this purpose, we analyzed the changes in microbiome in a group of PD subjects treated for six months with FPP compared to a control group treated with placebo. These effects were also evaluated with biochemical and clinical tests able to accurately describe the inflammatory and oxidative state (ox-inflammation biomarkers) and the evolution of disease [47].

METHODS

Study design and patient selection: This randomized, single-center, double-blind, cross-over, and placebo-controlled study was approved by the ethics committee of Vicenza (Italy) and was conducted on 39 volunteer PD subjects, 18 females (52 - 72 years) and 21 males (57 - 79 years). Twenty subjects were treated for 180 days with placebo (C = control group) while 19 were treated with FPP (V = verum group) consisting of 9 g/day Immun'Age (Named, Lesmo, Italy). After six months, the treatments were exchanged: the patients who formed group C in the first 180 days were treated with FPP in the second semester while group V patients of first semester were treated with placebo in the second. Each subject was observed in 3 visits: initial (T0, baseline), after 6 months (T180 cross-over visit) and after one year (T360, final visit). Four groups of data were thus obtained: the pair T0-T180 in patients initially treated with FPP or placebo (groups V1 and C1 respectively) and the pair T180-T360 in patients treated after with FPP or placebo between T180 and T360 (groups V2 and C2) (Table 1).

Inclusion criteria were a diagnosis of PD without fluctuations in stage 1-2 of the Hoehn-Yahr scale, being an age between 60 and 70 years, therapies with levodopa/carbidopa or levodopa/benderizine between 300 and 1200 mg/day and possible concurrent treatment with dopamine agonists. None of the therapies were modified during the study. After 1 year of study, PD patients were divided into two groups, which were treated for six months with FFP (V) and that with placebo (C). Each group was also evaluated by distinguishing two subgroups, depending on whether the treatment was done in the first (V1 and C1) or in the second 6 months (V2 and C2), assuming that it was not indifferent whether the patient had been treated before with either FFP or a placebo.

Table 1. Randomization and treatment scheme of study.

Group	Treatment	Number of subjects	Treatment 1st 180 days	Treatment 2nd 180 days
C	180 days placebo	39	all subjects treated with placebo for 180 days, in 1st or 2nd 180 days	
V	180 days verum	39	all subjects treated with verum for 180 days, in 1st or 2nd 180 days	
C1	V0 - V180 placebo	20	placebo	-
C2	V0-180 verum – V180-360 placebo	19	verum	placebo
V1	V0 - V180 verum	19	verum	-
V2	V0-180 placebo – V180-360 verum	20	placebo	verum

Exclusion criteria were a diagnosis of renal insufficiency (glomerular filtration rate <30 mL/min/m2). The enrollments and medical examinations were performed in Clinic Villa Margherita (Vicenza, Italy); all the subjects were randomized 1:1 to receive FPP or placebo.

Clinical evaluations: the clinical parameters were recorded through the Unified Parkinson Disease Rating Scale (UPDRS) as part I (non-motor symptoms), part II (activities of daily living), part III (motor symptoms) in phase on, part IV (motor fluctuations and dyskinesias), and through the Hoehn & Yahr scale (HY) (stage of disease), Parkinson Disease Fatigue Rating Scale (fatigue), global cognitive assessment (Montreal Overall Cognitive Assessment - MOCA) and assessment of frontal cognitive functions (Frontal Assessment Battery - FAB). An assessment of disease-related quality of life was also performed using the Parkinson Disease Quality of life (PDQ-8) scale.

Biochemical assays: The laboratory tests for microbioma and redox imbalance were performed all together in batches at the end of the study, with exception of the biochemical parameters of metabolic profile which was determined contextually to the visits T0, T180 and T360; the plasma, whole blood, urine, and feces samples for evaluation of the oxidative stress, inflammation and microbiome were instead frozen at -80 °C until analysis. The blood samples were collected in vacuum tubes with EDTA or without anticoagulant, as required by the different analytical methods.

All the parameters of biochemistry profile were analyzed on AU 5800 and DXI 800 platforms (Beckman coulter): glucose (G), urea (BUN), creatinine (CR), total cholesterol (CHOL), HDL, LDL, triglycerides (TG), CPK, uric acid (UA), total bilirubin (BT), AST, high sensitivity C-reactive protein (hsCRP), sodium (NA), potassium (K), ALT, GGT; the hematological parameters, erythrocytes (RBC), leukocytes (WBC), hemoglobin (Hb), platelets (PLT), hematocrit (HCT), leukocyte differential count (NE, LF, MONO, EOS, BASO) were measured on Unicell DXH 900 platform (Beckman coulter).

The ox-inflammation biomarkers, reactive oxygen metabolites (dROMs), and antioxidant biological potential (BAP) were determined by colorimetric assays (Diacron): the redox balance (Index) was then calculated according to a previously proposed algorithm [14]; the components of non-enzymatic endogenous antioxidant barrier, total (GSH + GSSG), oxidized (GSSG) and reduced glutathione (GSH) were determined in EDTA whole blood by HPLC (mod. 1100 Agilent, Santa Clara, CA) with specific kit (Eureka Lab Division, Chiaravalle, Italy). The protein oxidation serum marker 3-nitrotyrosine (3NT), and the nucleobases oxidation markers urinary 8-hydroxy-deoxy-guanosine (8OHdG) and 2-deoxy-guanosine (2dG) were

analyzed by HPLC with specific kit (Eureka Lab Division): 8OHdG and 2dG have been then expressed as ratio to urinary uric acid and creatinine concentrations. All the oxidation biomarkers were analyzed by CSOx lab (Vicenza, Italy) and Oxilab (Villafranca of Verona, Italy).

Statistical analysis: The data of biochemical profile and ox-inflammation biomarkers were processed to obtain means, standard deviations (SD), medians and inter-quartile ranges (IQR). Due to the asymmetry in distribution of most parameters, all the descriptive statistics have been discussed as median and IQR. The data obtained after 180 days of each treatment (at T180 or T360) were compared to the baseline (T0 or T180), either with the parametric t-tests and Wilcoxon non-parametric tests for dependent, with significance value of $p<0.05$.

For all the biochemical tests, the variations were also evaluated by comparison with the respective critical delta values (CDV%), according to Fraser and Harris [48-49].

Microbiome assay: the analyses were performed by BMR Genomics (Padova, Italy) by sequencing 16S rRNA gene. 100 mg of faeces were collected with preservative buffer (Beaver, Suzhou, China) and lysed using 750 µl of Bead Solution e 60 µl of C1 solution PowerFecal® (Qiagen, Germantown, USA) and about 200-300 µl of 0,1 mm zirconia-silica beads (Biospec, Bartlesville, USA). After 10 minutes of 65°C incubation, faeces were shaken with tissue lyser for 10 minutes at 25 Hz. After 1 min of centrifuge at 13000g, 200 ul of lysate were used as input material for Cador Pathogen 96 QIAcube HT Kit (Quiagen) on Qiacube HT instrument.

The V3-V4 regions of the 16S ribosomal RNA gene were amplified using Illumina tailed primers Pro341F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGAGGCAGCA-3') and Pro805R (5'-GTCTCGTGGGCTCGGA-GATGTGTATAAGAGACAGGACTACN-VGGGTATCTAATCC-3') using Platinum Taq (Thermo Fisher Scientific Inc, USA) by means PCR (94° C for 1 2min, followed by 25 cycles at 94° C for 30s, 55° C for 30s, and 68 ° C for 4530 s, and a final extension at 68 °C for 7 min). PCR amplicons were purified by means of Agencourt AMPure XP Beads 0.8X (Beckman Coulter, Inc., CA, USA) and amplified following the Nextera XT Index protocol (Illumina Inc, CA, USA). The indexed purified amplicons were normalized by SequalPrepTM Normalization Plate Kit (Thermo Fisher Scientific Inc.) and multiplexed. The pool was purified with 1X Magnetic Beads Agencourt XP (Beckman Coulter, Inc.), loaded on the MiSeq System (Illumina, Inc.) and sequenced following the V3 - 300PE strategy.

The bioinformatics analysis of 16S data was performed by means of QIIME2 version 2020.2 [50]. Primers were removed using the q2-cutadapt plugin. Later, paired-end reads were subjected to quality analysis including denoising, merging, and chimera removal using the DADA2 plugin [51] implemented in QIIME 2 (dada2 denoise-paired with the following parameters trunc_len_f:260, trunc_len_r:245). The resulting table containing amplicon sequence variants (ASVs) [52] was subsequently filtered at 0.05% in order to remove low covered ASVs. Furthermore, q2-feature-classifier plugin [53] was applied for assigning taxonomy to ASVs, using trained OTUs at 99% from Silva version 132 and Green Genes version 13-8 databases [54]. Samples were rarefied to 21,795 reads before downstream alpha and beta diversity analyses, leading to the exclusion of one sample.

Ecological analyses were performed exploiting various QIIME2 diversity plugins. Observed OTUs, Shannon, evenness, and Faith's phylogenetic diversity metrics [55] were chosen to evaluate the samples alpha diversity. The Kruskal–Wallis test was used as a non-parametric statistical test to assess pairwise differences. To investigate the microbial dissimilarity among the groups,

the time points and considering both together, four different beta diversity metrics, including Bray–Curtis, Jaccard, weighted Unifrac, and unweighted Unifrac, and their corresponding principal coordinate analysis (PCoA) were computed. PERMANOVA test with 999 permutations was applied for assaying beta-diversity significance (beta-group-significance).

ANCOM plugin implemented in Qiime2 was used to evaluate significant differences in taxa abundance at various levels (family, genus, species and ASV) after the proper pseudo-count addition to the various table (qiime composition add-pseudocount) [56].

Differential abundance analysis was also performed with DESeq2 [57]. Briefly, "Phyloseq" R package was used firstly to import Qiime2 artifacts in the R environment, including ASVs filtered table, phylogenetic tree with a tip for each ASV, ASVs taxonomy, clinical and experimental samples information [58]. Then, on the Phyloseq object, the "DESeq2" function was applied. This function first estimates the taxon-wise dispersion by maximum likelihood estimation, then fits the dispersion trend combining all individual estimates, and finally shrinks the taxon-wise dispersion estimates towards the values predicted by the trend curve using an empirical Bayes approach. Differential abundant taxa were selected on the multiple samples, in each taxonomic level (family, genus and species), with adjusted p value <0.05 as cut-off. "Pheatmap" and "ggplot2" R packages were used for generating heatmap and dot plot data visualizations.

RESULTS

The survey scales scores recorded at the start and end of each treatment were compared in groups C (C1+C2) and V (V1+V2) with both Wilcoxon and t-test. In a similar way, these analyses showed a greater number of significant ameliorative changes in V than in C group (Table 2).

Table 2. Statistical analysis of scores of survey scales recorded in control (C) and verum (V) subjects at the start and end of each treatment compared with both t-test and Wilcoxon test: the significant differences (p-values) are highlighted in bold.

Evaluation scale	Index of better change	t-test		Wilcoxon test	
		Group C	Group V	Group C	Group V
FAB	increase	0,562	0,453	0,779	0,82
H&Y	decrease	0,057	1,000	n.a.	n.a.
MOCA	increase	0,734	**0,015**	0,704	**0,025**
PDQ-8	decrease	0,055	**0,035**	0,056	**0,029**
UPDRS I	decrease	**0,035**	**0,005**	**0,041**	**0,003**
UPDRS II	decrease	0,515	**0,023**	0,689	**0,001**
UPDRS III	decrease	0,557	0,080	0,660	0,103
UPDRS IV	decrease	0,199	0,061	0,174	**0,031**
UPDRS tot	decrease	0,106	**0,001**	0,075	**0,001**

In group V, the rating scales with statistically significant improvements were much more than in group C.

For all groups, neither in the period T0-T180 nor in T180-T360 the medians of biochemistry, hematology and ox-inflammation parameters have shown clinically significant differences because the threshold of the respective CDV% was never exceeded. The data concerning the whole groups C and V are reported in Figure 1.

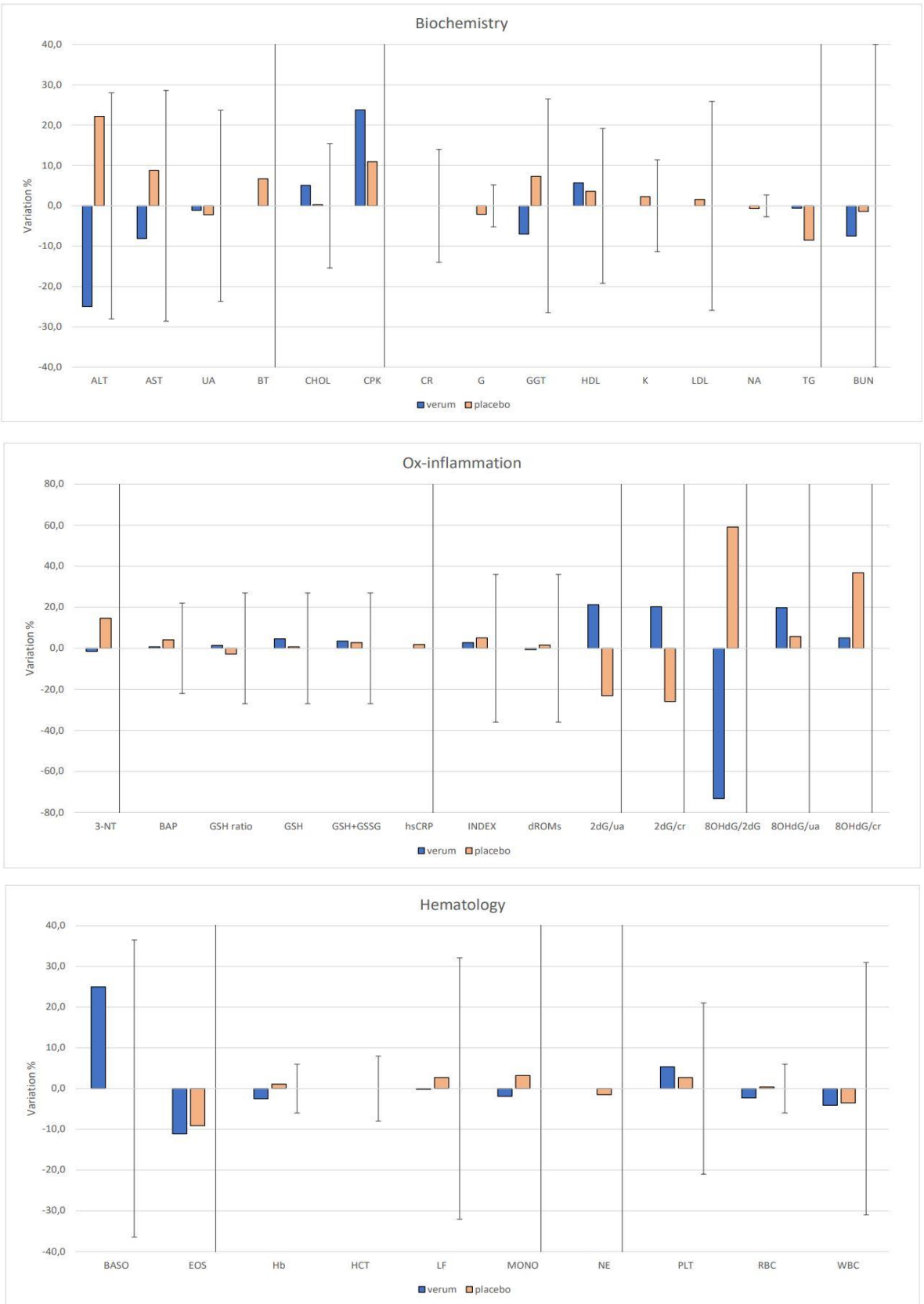


Figure 1. The percentage variation for each biochemical, hematological, and ox-inflammation parameter measured at the end vs. start of the study in control (C, pink columns) and in verum group (V, blue columns) are represented. No parameter showed significant clinical variations as the changes were always lower than the respective negative (⊥) and positive (⊤) CDV% values calculated from the respective analytical and intra-subject biological variabilities.

Instead, statistics have shown significant variations for 2dG and 8OHdG/2dG ratio in group V only, both between T0-T180 (V1) and T180-T360 (V2). Even in group C considered as a whole (C1+C2), a significant difference was highlighted for 8OHdG/2dG ratio ($p = 0.046$); instead, when analyzing the data separately, in group C1 no difference was shown, while in group C2 a significant difference for 8OHdG/2dG ($p = 0.032$) was found. In microbioma, the differential abundance analysis, performed with DESeq2 pipeline on various taxonomic levels (from family to species), highlighted the presence of some significant taxa.

In group C1, the family Mogibacteriaceae was found to be statistically significant, as well as the genus Haemophilus and the species Prevotella copri. On the other hand, comparing V1, no family was highlighted as differentially abundant. Instead, differences were detected at genus (Megasphaera) and species levels (Ruminococcus callidus, Megasphaera sp. and Clostridium hathewayi).

Similarly, no difference was found at the family level when comparing group C2, while abundances of five genera, Eubacterium and Megasphaera, with highest log Fold-change (logFC > 2.5), Escherichia, Clostridium and Prevotella, and five species Prevotella sp., Megasphaera sp., Escherichia coli, Bacteroides fragilis and Eubacterium biforme were significantly different.

At the family level, no significant differences have been highlighted in V2. Nevertheless, Eubacterium genus has been found strictly most abundant in T180, while evaluating species, in T360 has been found poorer in Bifidobacterium sp. and Ruminococcus gnavus, and richer in Bacteroides sp, Prevotella copri, Bacteroides plebeius and Eubacterium biforme.

Overall, after 180 days of treatment with FPP, the qualitative comparisons of microbiome data, considering phyla, families, genera, and species, led only to 41 non-univocal cases with statistical significance.

DISCUSSION

Some studies on chronic degenerative diseases such as thalassemia, cirrhosis, diabetes as well as aging have shown that FPP can act favorably on the immune and vascular systems through an anti-inflammatory action and a modification of the biomarkers of oxidative stress damage [59-62]. For these positive direct and indirect effects on health, FPP is considered a functional food [63-64]. Studies *in vitro* attempted to explain the molecular mechanisms of papaya extract but have not yet led to univocal conclusions [65-67].

The recent interest of the scientific community for the intestinal microbial flora, its genome (microbiome) and involvement in pathological conditions, has led to interesting observations in neurodegenerative diseases [68] and to hypothesize the existence of a connecting axis between brain and gut microbiota. Particularly in PD, it has been demonstrated that dysbiosis and the consequent inflammation and oxidative alteration may be pathogenetic factors [69-70]. Therefore, the advantages shown by FPP supplementation in some other pathologies suggested the possibility that, even in PD, fermented papaya might be acting as an ameliorative pre-biotic factor for intestinal microbial flora.

Our study does not seem to confirm however this hypothesis. Considering that in PD, Actinobacteria, Bacteroidetes and Firmicutes typically decrease and Proteobacteria increase, the increases in the former and the decrease in the latter can be considered as useful variations, while the opposite can be considered as expected variations. Our results were inconclusive on this matter because the variations were as follows: among the group V, 12% were useful changes and 49% expected changes; among the group C, 24% were useful changes and 15% expected changes.

Despite the limited number and non-homogeneous variations observed, since treatment with fermented papaya seems to lead to fewer useful variations and a

greater number of expected variations than placebo, assuming unlikely that the latter determine variations in microbiota, treatment with FPP showed no improvements.

The analysis of quantitative differences between phyla shows that the percentage in Proteobacteria were slightly higher in group V (+ 4.6%) than in C (+ 1.6%), as if the placebo was more beneficial in slowing the disease-related increase of this phylum (and this is unlikely). Because Actinobacteria, Bacteroidetes and Firmicutes, on the other hand, did not vary in either group considered, it can be concluded that there is no evidence that FPP treatment induces quantitative changes in the microbiota compared to placebo.

Because none of the biochemical parameters showed changes as greater as the respective CDV%, it must be concluded that the measured variations were lower than the expected intra-individual biological variability and therefore not clinically significant. However, the relevant and statistically significant differences in the 8OHdG/2dG ratio between V and C (-73% vs +59% compared to the baseline respectively) seems to demonstrate that in the control group, the oxidation of nucleic acids increased, while in verum decreased. Further differences, but less evident and not statistically significant, are observed for other parameters of ox-inflammation such as total and reduced glutathione, which increase after 180 days in V, 3NT that decreases in V and increases in C, and d-ROMs that do not change in V while increase in C. All together, the data seems to indicate that when taking FPP, the level of free radicals does not increase, the antioxidant barrier is strengthened and oxidative damage on proteins and especially on DNA decreases. Decrements of 2dG in C and increments in V suggests that, in verum group, there is less need to replenish the intracellular nucleotide pool because the oxidation of nitrogenous bases has decreased; furthermore, the little increase of 8OHdG/creat ratio in V than in C seems to indicate that the nucleic acid oxidation, which is a feature of this disease, progress in V less significantly than in C. Finally, the lower oxidation of nitrogenous bases and their consequent catabolism to uric acid could explain the greater 8OHdG/uric acid ratio in V than in C.

With statistical significance, it has also been demonstrated that FPP treatment significantly decreased the 8OHdG/2dG ratio also in the control subgroup C2. Because these patients have been previously treated with verum in the first 180 days, this suggests a possible prolonged beneficial effect of FPP after the interruption of treatment.

Regarding the clinical evaluations of motor symptoms and cognitive functions of patients, when comparing group C and V, the benefits of FPP treatment become clear. Indeed, the treatment with placebo have improved only one of the 9 scales of motor symptoms evaluation considered while the treatment with verum have improved 5 of 9 scales, considering the t-test, and 6 of 9 scales, considering the Wilcoxon test.

It can be concluded that FPP treatment, whatever its mechanism of action, seems to induce significant improvement in overall cognitive functions and in motor and non-motor clinical parameters, in addition to improvements in the ox-inflammatory balance leading to a reduction in molecular oxidative damage.

CONCLUSION

A prolonged supplementation with FPP may enhance the antioxidant barrier, decrease the oxidation of nucleic acids, and induce significant improvement in clinical parameters and in quality life of PD patients. There is no evidence, however, that these effects are accompanied by significant qualitative or quantitative modifications of the intestinal microbial flora.

It is now not possible to exclude that a FPP treatment may act, for example, to improve the

permeability of the intestinal membrane or modifying the metabolism of some bacteria counteracting the leaky gut syndrome, and that just these pathways allow the observed improvements. Further investigation will therefore be necessary.

Abbreviations: 2dG: 2-deoxyguanosine, 3NT: 3-nitrotyrosine, 8OHdG: 8-hydroxy-deoxyguanosine, ALT: alanine-aminotransferase, AST: aspartate-aminotransferase, BAP: antioxidant biological potential, BASO: basophils; BT: total bilirubin, BUN: urea, CDV%: critical delta value, CHOL: total cholesterol, CPK: creatine phosphokinase, CR: creatinine, dROMs: reactive oxygen metabolites, EOS: eosinophils, FAB: Frontal Assessment Battery, FPP: fermented papaya preparation, G: glucose, GGT: gamma-glutamyl-transferase, GSH: reduced glutathione, GSSG: oxidized glutathione, Hb: hemoglobin, HCT: hematocrit, HDL: high density lipoproteins cholesterol, hsCRP: high sensitivity C-reactive protein, HY: Hoehn & Yahr scale, K: potassium, LDL: low density lipoproteins cholesterol, LF: linfocytes, MOCA: Montreal Overall Cognitive Assessment, MONO: monocytes, NA: sodium, NE: neutrophils, PD: Parkinson's disease, PDQ-8: Parkinson Disease Quality of life scale, PLT: platelets, RBC: erythrocytes, RNS: nitrogen reactive species, ROS: oxygen reactive species, TG: triglycerides, UA: uric acid, UPDRS: Unified Parkinson Disease Rating Scale, WBC: leukocytes.

Authors Contribution: Concept and design: GPN, OB. Analysis and interpretation: AB, LB, GB, ES. Data collection: AB, GPN. Writing the article: AB. Critical revision of the article: AB. Final approval of the article: all authors. Statistical analysis: AB, LB, GB, ES. Overall responsibility: GPN.

Competing Interests: The authors declare no conflict of interest.

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