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Interim report from a 2-year double-blind RCT testing fermented papaya preparation on immune enhancement, endothelial health and QOL in elderly adults

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ABSTRACT

Background: Aging is associated with alterations in oxi-inflammatory-immune profile, and endothelial cell dysfunction. Indeed, increased generation of free radicals as well as immunosenescence are hallmarks of the aging process and age-related diseases. In the past 12 years or so, data has been accumulated on fermented papaya preparation (FPP®) (Osato Research Institute, Gifu, Japan), a specific functional food with robust redox and immune regulator nutrigenomics effect. The aim of this 2-year ongoing study of which we report the first-year data, was to test FPP® in redox, endothelial, and immune markers.

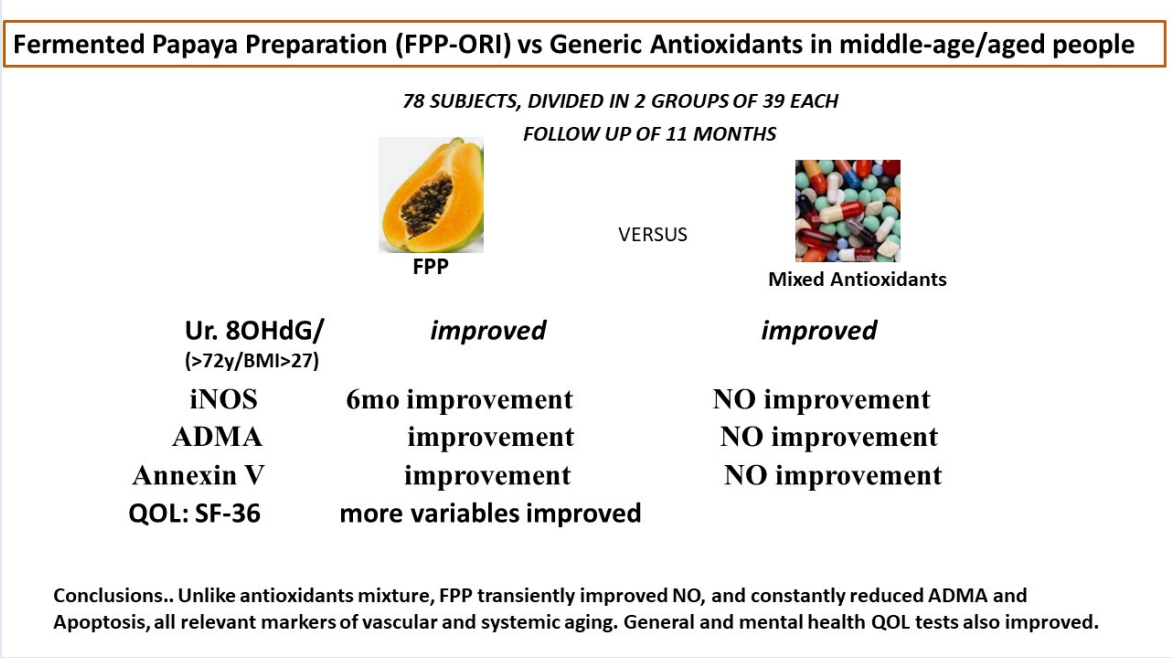
Methods: Study population. From a total of 106 subjects, we report the analyzed data referring to 78 clinically stable, healthy, community-dwelling males and females, aged 60 to 75 years. The study was conducted using a double-blind method with designated groups A and B to fulfill the two different treatments. The two treatments are as follows: Group A, also known as "FPP Group," was given one sachet two times per day containing 4.5g FPP®, along with one placebo capsule provided in the morning. Group B, also known as "AA Group," was given one papaya-flavored sachet two times

per day, along with one antioxidant mixture capsule in the morning. Morning blood samples were collected and tested for: Ultra-sensitive c-reactive protein (a highly sensitive ELISA), inducible nitric oxide synthase (iNOS) (by Human Nitric Oxide Synthase kit), asymmetric dimethylarginine, or ADMA, (a competitive enzyme-linked immunosorbent assay), apoptosis of PBMCs (by Annexin V staining) and MOS 36-item short-form health survey (SF-36) to assess quality of life. Screening and blood tests were carried out as follows: Visit I: Day 0 - Baseline, Visit II: Day 60, or 2 months, Visit III: 6 months, Visit IV: 11 months.

Results: Plasma iNOS levels were comparable among both groups at the beginning of the study. FPP®-treated subjects showed a significant increased level at Visits II and III (P<0.05 vs baseline and vs AA). ADMA values were not affected by AA supplementation whereas FPP® treatment was associated with a significant decrease beginning with observation during Visit III (P<0.05 vs baseline and vs AA administration). The FPP® intervention was associated with improvements among several domains of quality of life such as physical function, general health, and mental components (P < 0.01 vs baseline and vs AA group). There was also at significant and comparable positive effect for time on vitality shown in both AA and FPP® groups.

Conclusion: Unlike with the antioxidant treatment, the FPP® intervention yielded a transient decrease of ADMA, a decrease of iNOS and lower percentage in apoptotic PBMC. These results suggest that FPP®, by a more multifaceted, subcellular mechanism, as well as non-redox modulatory properties, was beneficially effective in regulating aging markers. These mechanisms are associated with a better SF-36 profile in support of FPP® as a candidate interventional functional food for health maintenance, and specifically in middle-age/elderly subjects.

Key words: Fermented Papaya Preparation, Nitric oxide, ADMA, apoptosis, antioxidants, SF-36



INTRODUCTION

Aging is associated with a remarkable modification in the inflammatory-immune profile, and is interrelated to a complex, and not yet fully unveiled, interplay between oxi-inflammatory mediators, immune cells, and endothelial cells taking place at an older age [1-3]. Nitric oxide (NO) represents the most significant vasodilatory effector produced by endothelial cells, which also act as a down-regulator factor for a number of pro-inflammatory cytokines, leukocyte, and thrombocyte accumulation, as well as atherosclerosis pathways [4-7]. In contrast, asymmetric dimethylarginine (ADMA), acts as an endogenous NOS inhibitor, and its increase represents a known risk factor for neurological diseases [8-10]. When the body is in excess of free radicals, the uncontrolled generation of NO itself, may transition to become detrimental and hinder the bioavailability of NO [11]. The ultimate result of this nitro-oxidative stress is cellular molecular injury, altered permeability of cell membranes, enhanced risk of age-related chronic diseases, and carcinogenesis [12-14]. However, the link between the two still has much unclear data [15]. The increased generation and immunosenescence of free radicals are established hallmarks of the aging process [16, 17]. Abnormalities of the CD4+/CD8+ ratio, as well as elevated neutrophil/lymphocyte ratio (NLR), have been reported as sensitive predicting markers for a poor prognosis in chronic inflammatory diseases, but also in acute bacterial and viral infections, including COVID-19, or in chemical-induced mutagenicity [18-23].

For the last 12 years, data accumulation has been compiled a fermented papaya preparation, a specific functional food with robust redox and immune regulator nutrigenomics effects [24-29]. We refer to the newest definition of functional food by the last FFC consensus illuminating the activity of bioactive compounds and the measurement of related biomarkers, [30].

The primary aim of the ongoing trial is to test the effect of a patented functional food, i.e., Fermented Papaya Preparation (FPP®)(Osato Research Institute, Gifu, Japan) prepared over a proprietary 8-month fermentation process on the following: nitric oxide synthase, asymmetric dimethylarginine (ADMA), and apoptosis of peripheral blood cells. Health-related quality of life (HRQOL) is an important concept to describe with regard to the subjective well-being of the general population and persons suffering from a disease. HRQOL is a significant patient-reported outcome [31]. The Short Form-36 (SF-36) is a widely utilized questionnaire assessing self-reported HRQOL in 8-month spans [31, 32]. Towards the end of 2 years, trials determining neutrophil-lymphocyte ratio and cytometry analysis of CD4+ and CD8+ cells had been completed, but further research is needed for further data collection. The study design and regulation were approved by the joint ethical committee of the ReGenera Research Association on January 19, 2020. Each subject recruited for the study was fully informed in all aspects of the process and followed the guidelines of the Declaration of Helsinki.

Study population: From a total of 106 subjects, here we report the analyzed data referring to 78 clinically stable, healthy, community-dwelling males and females (m/f: 38/40 respectively) aged 60 to 75 years, all negative for Covid-SARS2, and with BMI: 23.9 to 32.5 kg/m2. Mild dyslipidemia/borderline hyperglycemia was not considered as an exclusion criterion, if present, if the participant was not requiring pharmacological treatment. Exclusion criteria included several factors, including BMI ≥ 36, LDL >160 mg/dL, low Vitamin D marker (values would be normalized before trial), ApoE ε4 genotype, or any major cardiovascular event in the last 6 months. Any subjects with ailments related to endocrine system, immune system, respiratory system, hepatobiliary system, kidney, and urinary system, neuropsychiatric, blood, tumors and gastrointestinal diseases were omitted from the trial. Antibiotic use within 4 weeks of randomization, use of lipid-lowering drugs, antacids, proton pump inhibitors, corticosteroids

or sex hormones would also disqualify the individual from becoming a participant. Lastly, fish oils, multivitamins, probiotics, protein supplements, nutraceuticals, and/or substance abuse issues including nicotine/caffeine dependence, or high-risk drinking would also be a disqualifying factor. High-risk drinking is defined as consumption of 4+ alcohol-containing beverages on any day or 5+ alcohol-containing beverages per week for women and 7 or more alcohol-containing beverages per week for men [33]. None of the subjects were engaged in strenuous physical activity, but all were conducting normal physical activities (e.g., leisure walking, gardening, walking to the workplace etc.) therefore, this variable did not represent a parameter to differentiate study groups. Nonetheless, subjects had a pedometer installed on their mobile device and were requested to complete an average of 6000 steps per day without any pre-fixed performance target.

MATERIALS AND METHODS

Design of the study: Before entering the study, a pre-screening for thrombophilia was performed to exclude a hidden pro-thrombosis condition. The study was conducted in a double-blind methodology with two groups utilized: Group A, also known as Group FPP®, and Group B, also known as Group AAP. Group A was given 4.5g FPP®, 1 sachet two times per day, with one placebo capsule added in the morning. Group B was given a papaya-flavored sachet, 4.5g two times per day. One capsule in the morning contained an antioxidant mixture composed with 50mg epigallocatechin-gallate, 20mg anthocyanidins, 200mg trans-resveratrol, 80mg Ubiquinol, 5mg zinc, 100mg Centella asiatica extract, and 200IU Vitamin E.

Total duration of the study at completion will be 20 months (plus ≤3 weeks). Screening and blood tests were carried out as follows: Visit I: Baseline - Day 0, Visit II: Day 60 or 2mo.), Visit III: 6mo, Visit IV: 10-11mo., Visit V: 18mo., and Visit VI: 20mo.

METHODS

After an overnight fast, blood samples were withdrawn and examined in triplicate measures for a variety of tests, including serum AMDA, iNOS plasma concentration, Annexin V staining, and SF-36.

Morning blood samples were collected from patients after 12 hours of fasting. Subjects were asked to refrain from physical activity for at least 30 minutes prior to the blood draw. In addition to routine clinical laboratory tests, serum ADMA concentrations were analyzed by independent technicians who were blinded to group assignment and not involved in the treatment of the patients. Ultra-sensitivity c-reactive protein was assessed by a highly sensitive ELISA with a lower limit of detection of 0.01 mg/L [34]. The intra-assay CV was 2.4%, and the inter-assay CV was 10.6%. For the measurement of urinary 8-OHdG concentrations, also known as urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine (8OHdG), the monoclonal antibody recognizes 8-OHdG specifically. 8-OHdG levels were adjusted for urinary creatinine levels. Urinary 8-OHdG concentration was calculated as nanograms per milligram of creatinine [35]. The intra- and inter-assay coefficients of variation were 4.3% and 5.1%, respectively.

In order to assess the plasma concentration of iNOS, the Human Nitric Oxide Synthase 2 SEA837Hu reagent kit was used, with a detection range 0–1000pg/mL. Nitrate was first reduced to nitrite by nitrate reductase, then plasma levels of nitrite were assessed using spectrophotometry through the Griess reaction. The Griess reagent consists of a 1:1 mixture of 0.2% N-1 naphthyl-ethylene-diamine and sulfanilamide in 5% phosphoric acid. The Azo dye turns red violet in the presence of nitrite and is assessed by the spectrophotometer at 540nm [36].

A commercially available competitive enzyme-linked immunosorbent assay (ELISA) kit for ADMA (DLD Diagnostika GmbH, Hamburg, Germany), with a sensitivity 0.01µmol/L and inter-assay coefficients of variation of less than 3%, were used to assess endothelial dysfunction in all individuals [37]. After blood sampling, serum was separated and stored at −80 °C until further analysis. Annexin V staining was performed using PE-conjugated anti-annexin V antibody (eBioscience) in annexin V binding buffer (10 mM HEPES [pH7.4], 140 mM NaCl. 2.5 mM CaCl2) at RT for 15 min. DAPI (4',6-diamidino-2-phenylindole; Sigma-Aldrich) staining was utilized for excluding dead cells and apoptotic analysis [38]. Frequencies of apoptotic cells were computed by using BD LSRFortessa (BD Biosciences, San Jose, CA, USA). For Immune cell population profiles, PBMCs were isolated from the whole blood by density-gradient centrifugation using Ficoll-Paque density gradient media (GE Healthcare, Munich, Germany). Subsets of PBMCs were profiled with the antibodies specific for cell surface markers for CD4+ and CD8+ (Multitest Biosciences, San Jose, CA, USA) in FACS buffer (0.1% bovine calf serum and 0.05% sodium azide in 1 × PBS phosphate buffered saline at 4°C/30 min) by collecting 30,000 total events over at least 10,000 lymphocytes. Profiles of each population were examined by flow cytometry using Cytomics FC 500 and CXP software (Beckman Coulter), whereas frequencies of apoptotic cells were assessed using BD Biosciences, San Jose, CA, USA [39].

The Short Form-36 Health Survey Questionnaire (SF-36) is a standardized and widely used tool for assessing health-related QOL worldwide. The SF-36 is predominantly used to assess quality of life. According to the World Health Organization definition, quality of life (QOL) is related to culture, value system by which they live, goals, expectations, standards, and priorities. Physical and mental health, social relationships, personal beliefs, and the environment all affect the perceived QOL.

All tests were performed by SPSS, Version 22 (IBM Inc., Armonik, NY, USA). Results are presented with means ± standard deviations (SD) for continuous variables. We also used the baseline value of FPP® to analyze its time-course changes. Differences between study participants were assessed by t-tests and Chi^2-tests, with a critical alpha level of 0.05 being utilized for all analyses.

RESULTS

50% of the study seniors (n=39) had normal body mass index (18.5–24.9 kg/m2), 39.7% (n=31) were classified as overweight (≥25 and ≤29.9 kg/m2) and 10.2.% (n=8) subjects were classified as Class I obese (30 to < 35 kg/m2). Urinary 8-OHdG values appeared to be widely overlapping between the two groups at baseline and along the study (fig 1).

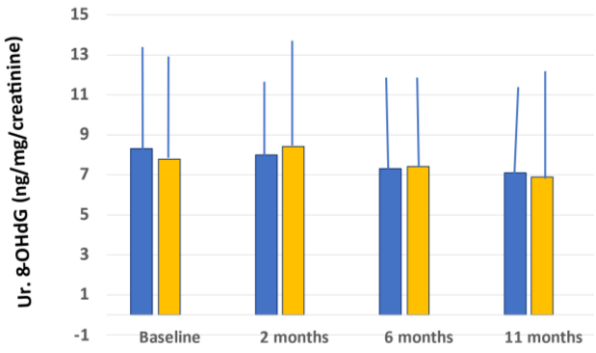


Figure 1. Time-course profile of urinary 8OHdG level in AA supplemented group (blue bars) and in FPP®-supplemented group (orange bars). No significant change appeared.

However, while plotting these values, subjects >72 years old (regardless of BMI), or all subjects with BMI >27 kg/m2 (regardless of age), showed urinary 8OHdG values that were significantly higher than the overall baseline (P<0.05) and both treatments equally improved them (Fig 2, P<0.05).

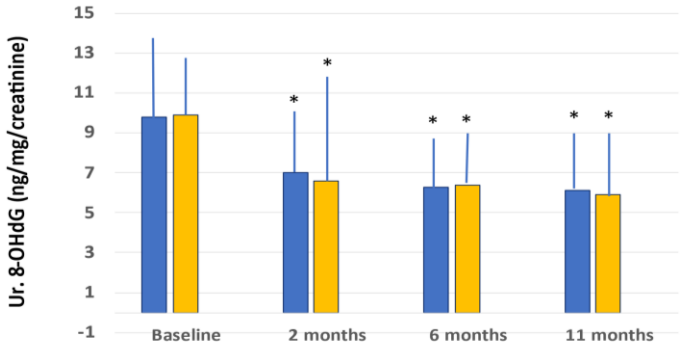


Figure 2. Time-course profile of urinary 8OHdG levels in AA supplemented group (blue bars) and in FPP®-supplemented group (orange bars) in the cluster of subjects > 72 years or with BMI >27. Both treatments revealed a significant decrease as compared to baseline (significance read at P<0.05).

Plasma iNOS levels were comparable among both groups at the beginning of the study (Fig 3). This concentration kept a constantly stable profile during the study period under AA treatment. However, FPP® treated subjects showed significant increased levels at the 2- and 6-month check (P<0.05 vs baseline and vs AA). No specific age, BMI, or gender correlation appeared in separate analysis (was not revealed). However, at the 11-month check, iNOS concentration returned to baseline-comparable values. No significant change in dietary habits occurred between 6- and 11-month observation.

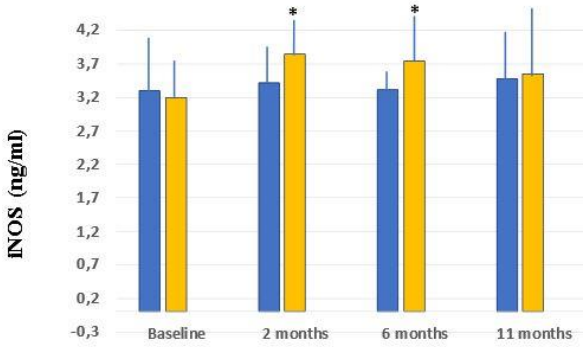


Figure 3. NOS plasma levels during AA (blue bars) and FPP® (orange bars) supplementation over 11-month follow-up. FPP® supplementation brought about a transient, but significant increase at 2 and 6 months (*P<0.05 vs baseline and AA administration).

Similarly, ADMA values seemed not to be affected by AA supplementation (Fig. 4), whereas FPP® treatment was associated with a significant decrease beginning from 6-month observation (P<0.05 vs baseline and vs AA administration).At the baseline, there was no significant difference between the two groups in the frequencies of apoptotic cells in the blood (Fig. 5). After the 6th month of trials, as compared to AA group which showed a gradual significant increase (P<0.05 vs baseline), FPP®-supplemented group maintained a significantly stable lower percentage (P <0.01 vs AA group values obtained at 6 and 11 months).

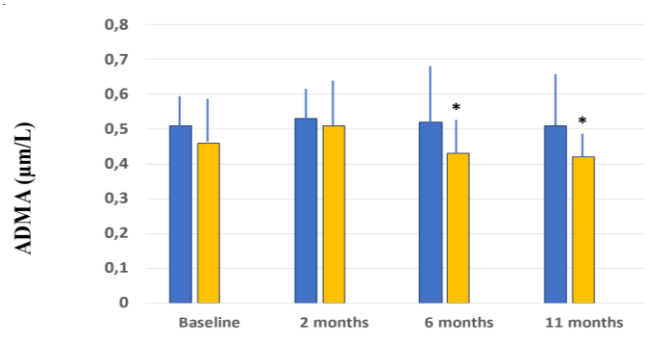


Figure 4. Time-course changes of plasma ADMA level in AA supplemented group (blue bars) and in FPP®-supplemented group (orange bars). Unlike AA supplementation, FPP® administration revealed a significant decrease at 6- and 11-month observation (*P<0.05 vs baseline and AA group).

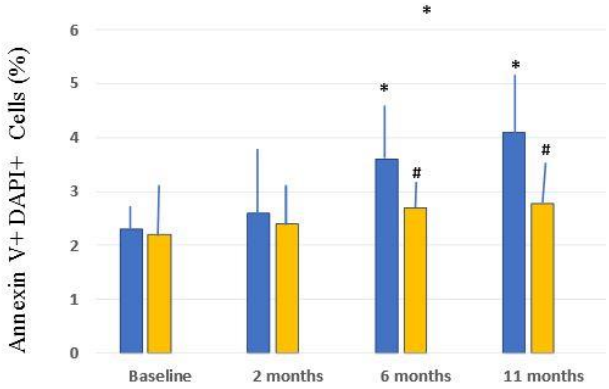


Figure 5. Frequency of apoptotic cells (Annexin V+ DAPI+) at baseline, 2, 6 and 11 months. Data presented as means ± SEMs. No significant differences between AA group and FPP® group at baseline appeared as measured by an unpaired t-test, and those measured afterwards were assessed by linear model adjusted to the baseline value. (*P< 0.01 vs baseline; #P<0.05 vs AA group at 6 and 11 months)

The FPP® intervention was associated with improvements or trends toward improvement among several domains of quality of life (Fig. 6). Physical function, general health, and mental components of SF-36 were significant ($p < 0.01$ vs baseline and vs AA group). There was also a significant, positive trend for effect and time on vitality in AA and FPP® groups at a comparable rate. The other variables were observed without affect by either treatment.

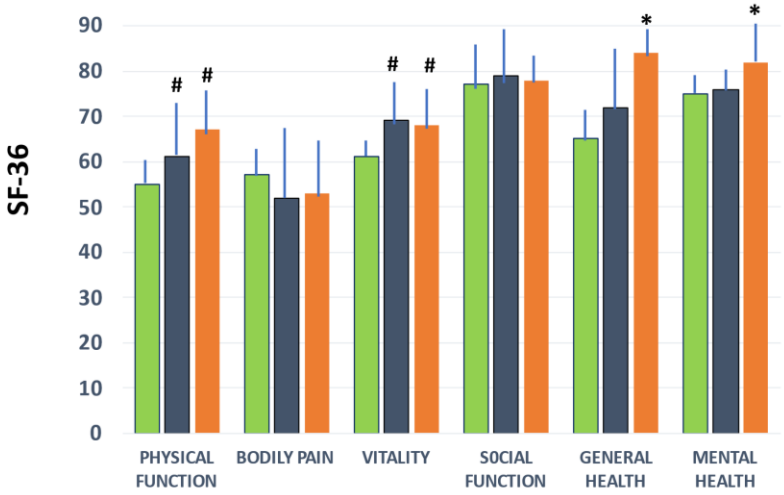


Figure 6. Short Form-36 Quality of Life questionnaire. Green Bar: pooled baseline values; Blue bar: values after antioxidant mixture at 11-month follow-up; Orange bar: values after FPP® at 11-month follow-up. (#P<0.01 vs baseline: *P<0.05 vs baseline and vs AA group.

DISCUSSION

Prior studies show that urinary levels of 8OHdG tend to be higher with aging processes, environmental toxin exposure, and observed in overweight individuals [40-45]. Thus, it was not surprising that the values of this marker were rather scattered in our wider range population and no effect of either interventions could be observed. However, when combining aged and overweight subjects, significantly higher values were observed and both interventions yielded a significant improvement. Endothelial dysfunction is a crucial process preceding the onset and progression of cardiovascular diseases and during aging growth [46-48]. This disorder is invariably associated to an overall systemic redox imbalance, inflammatory deregulatory pathways and accelerated senescence of vascular endothelial cells [49-51].

Naturally, endothelial physiology relies on a dynamic interplay of NO and ADMA. 20 years ago, it had been reported that ADMA inhibition of NO synthesis may negatively affect cerebral blood flow (CBF), thus contributing to cognitive decline including nerve degeneration [52]. Recently, the relationship of CBF impairment and NO signaling has been established by functional MRI techniques where the administration of NO synthesis inhibition in healthy subjects can decrease CBF in multiple brain areas by an 11% significance [53]. Belpomme's group [54], successfully treating a group of patients suffering from electro-hypersensitivity syndrome, clearly proved the clinical benefit of this specific FPP® when paired with a redox system and an increase in intracerebral tissue. Pulse metric index in the temporal lobes was measured by ultrasonic cerebral tomosphygmography (UCTS). In our present study, while antioxidant and FPP® exerted a comparably beneficial antioxidant effect, only FPP® showed a significant decrease in ADMA while also enhancing NO synthase activity at 2 and 6 months. We currently don't have a reasonable explanation of the transient, albeit significant, nature of this latter effect. A limitation noted in the protocol was to purposefully not schedule and/or monitor specific exercise workloads, therefore it would be possible to objectively assess the dependent variable. It is well known that following increased activity of endothelial nitric oxide synthase, NO synthesis rises in association with muscle cell contractions [55]. We may assume that untrained participants shortly after paying attention to their physical exercise by the pedometer, had an appreciable biological response of iNOS, but later the perceived intensity faded as did the NO response. This hypothesis may find support from the recent data from Shannon et al. [56], showing that during endurance exercise, a potential reduction in NO bioavailability may occur in elderly. To note, Casey et al. [57] has shown that vasodilator kinetics in exercising muscle are altered in aged population, presumably due to a inhibited NO signaling with a 45% decrease of the contribution of NO to exercise-induced hyperemia, as also clarified by Schrage et al. [58].

It should also be considered in a further analysis of data that the effects of FPP® may vary with the age of participants, considering the different physiologies of subjects' upper gastrointestinal tract [59]. On the other hand, neuronal nitric oxide synthase (nNOS) and iNOS are also located at brain level, and thus may be playing a role in the QOL perception. SF-36 analysis showed that variables such as physical function and vitality were comparably improved by both treatments, but only FPP® brought about a significant change in general well-being and mental health. Based on the data of Belpomme's group [54], we cannot rule out the perceptions of advancement in his study that are in line with our observed beneficial SF-36 response. On a cautious note, it must be mentioned that excess NO reacts with superoxide anions to produce peroxynitrite, a catalyst in the uncoupling of endothelial nitric oxide synthase

(eNOS), which perpetuates vascular oxidative stress. However, when superoxide anions are under control, as exerted by FPP® [60, 61], this phenomenon is negligible. In study conducted by O'Gallagher et al. a vital physiological role of nNOS is represented by its regulatory effect in modulating regional CBF and neurophysiological networking in the hippocampus, a key region controlling cognitive and memory functions and affected during neurodegenerative illnesses [62].

Among the wide array of immune system regulators, such as dietary [63, 64] and gut related regulators [65-71], oxidative stress is undoubtedly altering immune metabolism and detrimentally affecting the T-lymphocyte phenotype. In this context, apoptosis is one of the consequences subject to an imbalanced redox system at the mitochondrial respiratory chain level, attacking proteins and lipids, and overwhelming the biological restoration capacity. This degeneration ultimately accelerates aging and age-related diseases. Against the lack of an antioxidant effect, the FPP® intervention yielded a lower percentage in apoptotic PBMCs at 6 and 11 months. This suggests that FPP®, by a multifaceted, subcellular mechanism, as well as non-redox modulatory properties, played by its fermentation-derived moieties, proving effective in beneficially regulating programmed cell death, which may have a variety of effectors [72, 73]. The discipline of functional food intervention in aging and cancer is indeed an arena of very promising research studies [74-79]

In conclusion, this data represents the first batch of a larger study providing more data focusing on immune markers, a key factor in aging. The decrease in apoptosis observed in the present study needs to be tested against the frequency of CD14-positive PBMCs, which is mainly expressed on the surface of monocytes and easily induced by oxidative stress to remove apoptotic cells. Given the present limitations, in the quest for science-based strategies for longevity and chronic disease risk prevention, the present data supports FPP® as a candidate for interventional functional food, where functional food is defined as a specific bioactive- and biomarker-endowed, non-GMO, clinical trial-tested dietary element for health maintenance and an adjuvant weapon in medical treatments.

Abbreviations: iNOS: Inducible nitric oxide synthase; ADMA: Asymmetric dimethylarginine; PBMCs: Peripheral Blood Mononuclear Cells; SF-36: 36-item short form health survey; FPP®: Fermented Papaya Preparation; BMI: Body Mass Index; 8OHdG: 8-oxo-7,8-dihydro-2'-deoxyguanosine

Contribution: LA, OM discussed on the research plan, LA, RS and BM followed the clinical aspects FH and AC discussed and evaluated nutritional aspects, AA, OM and BM discussed and evaluated the biochemistry.

Conflict of interest: authors declare no personal or institutional conflict of interest.

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REFERENCES

1. Nicolson G, Breeding P, Settineri R, Ferreira de Mattos G.Aging and chronic illnesses: Membrane Lipid Replacement for restoring mitochondrial function and reducing fatigue, pain, and other symptoms in aged individuals. Bioactive Compounds in Health ad Disease, 2020; 3(10):194-203 DOI: https://doi.org/10.31989/bchd.v3i10.749
2. Kaufman-Shriqui V, Navarro DA, Salem H, Boaz M.Mediterranean diet and health – a narrative review. Functional Foods in Health and Disease 2022; 12(9):479-487. DOI: https://www.doi.org/10.31989/ffhd.v12i9.989
3. Martínez de Toda I, Ceprián N, Díaz-Del Cerro E, De la Fuente M. The Role of Immune Cells in Oxi-Inflamm-Aging. Cells. 2021 1;10(11):2974. DOI:

https://www.doi.org/10.3390/cells10112974.

4. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007 Jan;87(1):315-424. DOI: https://www.doi.org/10.1152/physrev.00029.2006.
5. Förstermann U, Xia N, Li H. Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circ Res. 2017, 17;120(4):713-735. DOI: https://www.doi.org/10.1161/CIRCRESAHA.116.309326
6. Afanas'ev I. Superoxide and nitric oxide in senescence and aging. Front Biosci (Landmark Ed). 2009 Jan 1;14(10):3899-912. DOI: https://www.doi.org/10.2741/3499.
7. Gimbrone MA Jr, García-Cardeña G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circ Res. 2016 Feb 19;118(4):620-36. DOI: https://www.doi.org/10.1161/CIRCRESAHA.115.306301.
8. Marotta F, Mao GS, Liu T, Chui DH, Lorenzetti A, Xiao Y, Marandola P. Anti-inflammatory and neuroprotective effect of a phytoestrogen compound on rat microglia. Ann N Y Acad Sci. 2016 Nov; 1089:276-81. DOI: https://www.doi.org/.10.1196/annals.1386.033.
9. Jagadeesan AJ, Murugesan R, Vimala Devi S, Meera M, Madhumala G, Vishwanathan Padmaja M et al. Current trends in etiology, prognosis and therapeutic aspects of Parkinson's disease: a review. Acta Biomed. 2017 Oct 23;88(3):249-262. DOI: https://www.doi.org/10.23750/abm.v88i3.6063.
10. Barbagallo M, Marotta F, Dominguez LJ. Oxidative stress in patients with Alzheimer's disease: effect of extracts of fermented papaya powder. Mediators Inflamm. 2015; 2015:624801. https://www.doi.Radi R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proc Natl Acad Sci U S A. 2018 Jun 5;115(23):5839-5848. DOI: https://www.doi.org/1073/pnas.1804932115.
11. Morgunova GV, Klebanov AA, Marotta F, Khokhlov AN. Culture medium pH and stationary phase/chronological aging of different cells. Moscow University Biological Sciences Bulletin 2016, 72 (2), 47-51, DOI: https://www.doi.org/10.3103/S0096392517020109
12. Banerjee A, Sriramulu S, Catanzaro R, He F, Chabria Y, Balakrishnan B, Hari S. et al. Natural Compounds as Integrative Therapy for Liver Protection against Inflammatory and Carcinogenic Mechanisms: From Induction to Molecular Biology Advancement. Curr Mol Med. 2022 Mar 16. DOI: https://www.doi.org/10.2174/1566524022666220316102310.
13. Das A, Ganesan H, Sriramulu S, Marotta F, Kanna NRR, Banerjee A, He F. et al. A review on interplay between small RNAs and oxidative stress in cancer progression. Mol Cell Biochem. 2021 Nov;476(11):4117-4131. DOI: https://www.doi.org/10.1007/s11010-021-04228-9.
14. Rastmanesh R, Hejazi J, Marotta F, Hara N. Type 2 diabetes: a protective factor for prostate cancer? An overview of proposed mechanisms. Clin Genitourin Cancer. 2014 Jun;12(3):143-8. DOI: https://www.doi.org/10.1016/j.clgc.2014.01.001.
15. Santoro A, Bientinesi E, Monti D. Immunosenescence and inflammaging in the aging process: age-related diseases or longevity? Ageing Res Rev. 2021 Nov; 71:101422. DOI: https://www.doi.org/10.1016/j.arr.2021.101422.
16. Rodrigues LP, Teixeira VR, Alencar-Silva T, Simonassi-Paiva B, Pereira RW, Pogue R, Carvalho JL. Hallmarks of aging and immunosenescence: Connecting the dots. Cytokine Growth Factor Rev. 2021 Jun; 59:9-21. DOI: https://www.doi.org/10.1016/j.cytogfr.2021.01.006.
17. Hazeldine J, Lord JM. Immunesenescence: A Predisposing Risk Factor for the Development of COVID-19? Front Immunol. 2020 Oct 6; 11:573662. DOI: https://www.doi.10.3389/fimmu.2020.573662.
18. El-Gazzar AG, Kamel MH, Elbahnasy OKM, El-Naggar ME. Prognostic value of platelet and neutrophil to lymphocyte ratio in COPD patients. Expert Rev Respir Med. 2020 Jan;14(1):111-116. DOI: https://www.doi.org/10.1080/17476348.2019.1675517.
19. Semelka CT, DeWitt ME, Blevins MW, Holbrook BC, Sanders JW, Alexander-Miller MA. Frailty and Age Impact Immune Responses to Moderna COVID-19 mRNA Vaccine. Res Sq [Preprint]. 2022 Aug 1:rs.3.rs-1883093. DOI: https://www.doi.org/10.21203/rs.3.rs-1883093/v1.
20. Jaaban M, Zetoune AB, Hesenow S, Hessenow R. Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as novel risk markers for diabetic nephropathy in patients with type 2 diabetes. Heliyon. 2021 Jul 19;7(7):e07564. DOI: https://www.doi.org/10.1016/j.heliyon.2021.e07564.
21. Bedel C, Korkut M. Neutrophil-to-Lymphocyte Ratio and Covid-19. Shock. 2021 Nov 1;56(5):874. DOI: https://www.doi.org/10.1097/SHK.0000000000001712.
22. Yaduvanshi SK, Srivastava N, Marotta F, Jain S, Yadav H. Evaluation of micronuclei induction capacity and mutagenicity of organochlorine and organophosphate pesticides. Drug Metab Lett. 2012 Sep 1;6(3):187-97. DOI: https://www.doi.10.2174/1872312811206030006.
23. Bertuccelli G, Zerbinati N, Marcellino M, Nanda Kumar NS, He F, Tsepakolenko V, Cervi J et al. Effect of a quality-controlled

fermented nutraceutical on skin aging markers: An antioxidant-control, double-blind study. Exp Ther Med. 2016 Mar;11(3):909-916. DOI: https://www.doi.org/10.3892/etm.2016.3011.2016

24. Warang PP, Shinde NS, Umare VD, Deshmukh PV, Ghosh K, Madkaikar MR, Colah RB, Mukherjee MB. Role of Oxidative Stress and the Protective Effect of Fermented Papaya Preparation in Sickle Cell Disease. Hemoglobin. 2022 Jul;46(4):260-264. DOI: https://www.doi.org/10.1080/03630269.2022.2118603.
25. Marotta F, Chui DH, Jain S, Polimeni A, Koike K, Zhou L, Lorenzetti A et al. Effect of a fermented nutraceutical on thioredoxin level and TNF-alpha signalling in cirrhotic patients. J Biol Regul Homeost Agents. 2011 Jan-Mar;25(1):37-45.
26. Rimbach G, Park YC, Guo Q, Moini H, Qureshi N, Saliou C, Takayama K, Virgili F, Packer L. Nitric oxide synthesis and TNF-alpha secretion in RAW 264.7 macrophages: mode of action of a fermented papaya preparation. Life Sci. 2000 Jun 30;67(6):679-94. DOI: https://www.doi.org/10.1016/s0024-3205(00)00664-0.
27. Fibach E, Tan ES, Jamuar S, Ng I, Amer J, Rachmilewitz EA. Amelioration of oxidative stress in red blood cells from patients with beta-thalassemia major and intermedia and E-beta-thalassemia following administration of a fermented papaya preparation. Phytother Res. 2010 Sep;24(9):1334-8. DOI: https://www.doi.org/10.1002/ptr.3116.
28. Marotta F, Pavasuthipaisit K, Yoshida C, Albergati F, Marandola P. Relationship between aging and susceptibility of erythrocytes to oxidative damage: in view of nutraceutical interventions. Rejuvenation Res. 2006 Summer;9(2):227-30. DOI: https://www.doi.org/10.1089/rej.2006.9.227.
29. Adany, A. Kanya, H. Martirosyan, D. Japan's health food industry: An analysis of the efficacy of the FOSHU system. Bioactive Compounds in Health and Disease 2021; 4(4):63-78. DOI: https://www.doi.org/10.31989/bchd.v4i4.795
30. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992; 30(6):473–83.
31. Lins L, Carvalho FM. SF-36 total score as a single measure of health-related quality of life: Scoping review. SAGE Open Med. 2016 Oct 4; 4:2050312116671725. DOI: https://www.doi.org/10.1177/2050312116671725.
32. Carpenter RW, Treloar Padovano H, Emery NN, Miranda R Jr. Rate of alcohol consumption in the daily life of adolescents and emerging adults. Psychopharmacology (Berl). 2019 Nov;236(11):3111-3124. DOI: https://www.doi.org/10.1007/s00213-019-05262-8.
33. Lane MM, Lotfaliany M, Forbes M, Loughman A, Rocks T, O'Neil A, Machado P, Jacka FN, Hodge A, Marx W. Higher Ultra-Processed Food Consumption Is Associated with Greater High-Sensitivity C-Reactive Protein Concentration in Adults: Cross-Sectional Results from the Melbourne Collaborative Cohort Study. Nutrients. 2022 Aug 12;14(16):3309, DOI: https://www.doi.org/10.3390/nu14163309.
34. Graille M, Wild P, Sauvain JJ, Hemmendinger M, Guseva Canu I, Hopf NB. Urinary 8-OHdG as a Biomarker for Oxidative Stress: A Systematic Literature Review and Meta-Analysis. Int J Mol Sci. 2020 May 26;21(11):3743. DOI: https://www.doi.org/10.3390/ijms21113743.
35. Yoon HJ, Chay KO, Yang SY. Native low-density lipoprotein increases the production of both nitric oxide and reactive oxygen species in the human umbilical vein endothelial cells. Genes Genomics. 2019 Mar;41(3):373-379. DOI: https://www.doi.org/10.1007/s13258-018-00777-4.
36. Bouras G, Deftereos S, Tousoulis D, Giannopoulos G, Chatzis G, Tsounis D, Cleman MW, Stefanadis C. Asymmetric Dimethylarginine (ADMA): a promising biomarker for cardiovascular disease? Curr Top Med Chem. 2013;13(2):180-200. DOI: https://www.doi.org/10.2174/1568026611313020007.
37. Atale N, Gupta S, Yadav UC, Rani V. Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. J Microsc. 2014 Jul;255(1):7-19. DOI: https://www.doi.org/10.1111/jmi.12133.
38. Saxena A, Dagur PK, Biancotto A. Multiparametric Flow Cytometry Analysis of Naïve, Memory, and Effector T Cells. Methods Mol Biol. 2019; 2032:129-140. DOI: https://www.doi.org/10.1007/978-1-4939-9650-6_8.
39. Guo C, Wei J, Yang J, Xu J, Pang W, Jiang Y. Pomegranate juice is potentially better than apple juice in improving antioxidant function in elderly subjects. Nutr Res. 2008 Feb;28(2):72-7. DOI: https://www.doi.org/10.1016/j.nutres.2007.12.001.
40. Kocael A, Erman H, Zengin K, Kocael PC, Korkmaz GG, Gelisgen R, Taskin M, Ersan Y, Uzun H. The effects on oxidative DNA damage of laparoscopic gastric band applications in morbidly obese patients. Can J Surg. 2014 Jun;57(3):183-7. DOI: https://www.doi.org/10.1503/cjs.008113.
41. Donati A, Cavallini G, Bergamini E. Effects of aging, antiaging calorie restriction and in vivo stimulation of autophagy on the urinary excretion of 8OHdG in male Sprague-Dawley rats. Age (Dordr). 2013 Apr;35(2):261-70. DOI: https://www.doi.org/10.1007/s11357-011-9346-x.

42. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009 Apr;27(2):120-39. DOI: https://www.doi.org/10.1080/10590500902885684.
43. Propson NE, Roy ER, Litvinchuk A, Köhl J, Zheng H. Endothelial C3a receptor mediates vascular inflammation and blood-brain barrier permeability during aging. J Clin Invest. 2021 Jan 4;131(1):e140966. DOI: https://www.doi.org/10.1172/JCI140966. PMID: 32990682; X
44. Tamae K, Kawai K, Yamasaki S, Kawanami K, Ikeda M, Takahashi K, Miyamoto T, Kato N, Kasai H. Effect of age, smoking and other lifestyle factors on urinary 7-methylguanine and 8-hydroxydeoxyguanosine. Cancer Sci. 2009 Apr;100(4):715-21. DOI: https://www.doi.org/10.1111/j.1349-7006.2009.01088.x.
45. Balta S. Endothelial Dysfunction and Inflammatory Markers of Vascular Disease. Curr Vasc Pharmacol. 2021;19(3):243-249. https://www.doi.org/10.2174/1570161118666200421142542.
46. Jia G, Aroor AR, Jia C, Sowers JR. Endothelial cell senescence in aging-related vascular dysfunction. Biochim Biophys Acta Mol Basis Dis. 2019 Jul 1;1865(7):1802-1809. DOI: https://www.doi.org/10.1016/j.bbadis.2018.08.008.
47. Castro-Ferreira R, Cardoso R, Leite-Moreira A, Mansilha A. The Role of Endothelial Dysfunction and Inflammation in Chronic Venous Disease. Ann Vasc Surg. 2018 Jan; 46:380-393. DOI: https://www.doi.org/10.1016/j.avsg.2017.06.131.
48. Culley MK, Chan SY. Endothelial Senescence: A New Age in Pulmonary Hypertension. Circ Res. 2022 Mar 18;130(6):928-941. DOI: https://www.doi.10.1161/CIRCRESAHA.121.319815.
49. Ungvari Z, Tarantini S, Kiss T, Wren JD, Giles CB, Griffin CT, Murfee WL, Pacher P, Csiszar A. Endothelial dysfunction and angiogenesis impairment in the ageing vasculature. Nat Rev Cardiol. 2018 Sep;15(9):555-565. https://www.doi.org/10.1038/s41569-018-0030-z.
50. Tesauro M, Mauriello A, Rovella V, Annicchiarico-Petruzzelli M, Cardillo C, Melino G, Di Daniele N. Arterial ageing: from endothelial dysfunction to vascular calcification. J Intern Med. 2017 May;281(5):471-482. DOI: https://www.doi.org/10.1111/joim.12605.
51. Selley ML. Increased concentrations of homocysteine and asymmetric dimethylarginine and decreased concentrations of nitric oxide in the plasma of patients with Alzheimer's disease. Neurobiol Aging. 2003 Nov;24(7):903-7. DOI: https://www.doi.org/10.1016/s0197-4580(03)00007-1
52. Carter KJ, Ward AT, Kellawan JM, Eldridge MW, Al-Subu A, Walker BJ, Lee JW et al. Nitric oxide synthase inhibition in healthy adults reduces regional and total cerebral macrovascular blood flow and microvascular perfusion. J Physiol. 2021 Nov;599(22):4973-4989. DOI: https://www.doi.org/10.1113/JP281975. Epub 2021 Oct 13.
53. Irigaray P, Garrel C, Houssay C, Mantello P, Belpomme D. 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. Functional Foods in Health and Disease, 2018; 8:122-144. DOI: https://doi.org/10.31989/ffhd.v8i2.406
54. Ferrari L, Vicenzi M, Tarantini L, Barretta F, Sironi S, Baccarelli AA, Guazzi M et al. Effects of Physical Exercise on Endothelial Function and DNA Methylation. Int J Environ Res Public Health. 2019 Jul 16;16(14):2530.
55. Shannon OM, Clifford T, Seals DR, Craighead DH, Rossman MJ. Nitric oxide, aging and aerobic exercise: Sedentary individuals to master's athletes. Nitric Oxide. 2022 Aug 1;125-126:31-39. DOI: https://www.doi.org/10.1016/j.niox.2022.06.002.
56. Casey DP, Ranadive SM, Joyner MJ. Aging is associated with altered vasodilator kinetics in dynamically contracting muscle: role of nitric oxide. J Appl Physiol. 2015 Aug 1;119(3):232-41. DOI: https://www.doi.org/10.1152/japplphysiol.00787.2014.
57. Schrage WG, Eisenach JH, Joyner MJ. Ageing reduces nitric-oxide- and prostaglandin-mediated vasodilatation in exercising humans. J Physiol. 2007 Feb 15;579(Pt 1):227-36. DOI: https://www.doi.org/10.1113/jphysiol.2006.124313.
58. Marotta F, Hayakawa K, Mikami Y, Morello P, Sugai M, Morita T. Relationship between gastrin cell number, serum, antral mucosa and luminal gastrin concentration and gastric acidity in antral atrophic gastritis. Gut. 2010 Mar;31(3):279-81. DOI: https://www.doi.org/10.1136/gut.31.3.279.
59. Marotta F, Koike K, Lorenzetti A, Jain S, Signorelli P, Metugriachuk Y, Mantello P, Locorotondo N. Regulating redox balance gene expression in healthy individuals by nutraceuticals: a pilot study. Rejuvenation Res. 2010 Apr-Jun;13(2-3):175-8.
60. Bertuccelli G, Marotta F, Zerbinati N, Cabeca A, He F, Jain S, Lorenzetti A, Yadav H, Milazzo M, Calabrese F, Tomella C, Catanzaro R. Iron supplementation in young iron-deficient females causes gastrointestinal redox imbalance: protective effect of a fermented nutraceutical. J Biol Regul Homeost Agents. 2014 Jan-Mar;28(1):53-63.
61. O'Gallagher K, Puledda F, O'Daly O, Ryan M, Dancy L, Chowienczyk PJ, Zelaya F, Goadsby PJ, Shah AM. Neuronal nitric oxide synthase

regulates regional brain perfusion in healthy humans. Cardiovasc Res. 2022 Mar 25;118(5):1321-1329. DOI: https://www.doi.org/10.1093/cvr/cvab155.

62. Mondal S, Padmakumari Soumya NP, Mini S, Sivan SK. Bioactive compounds in functional food and their role as therapeutics. Bioactive Compounds in Health and Disease. 2021; 4(3):24-39. DOI: https://www.doi.org/10.31989/bchd.v4i3.786
63. Ayivi R, Ibrahim SA, Colleran H, Silva R, Williams L, Galanakis C, Fidan H, Tomovska J, Siddiqui SA. COVID-19: human immune response and the influence of food ingredients and active compounds. Bioactive Compounds in Health and Disease. 2021; 4(6):100-148. DOI: https://www.doi.org/10.31989/bchd.v4i6.802
64. Martirosyan DM, Leem C. The bioactive compounds of probiotic foods/supplements and their application in managing mental disorders. Bioactive Compounds in Health and Disease. 2019: 2(10):206-220. DOI: https://www.doi.org/10.31989/bchd.v2i10.431
65. Nagpal R, Mainali R, Ahmadi S, Wang S, Singh R, Kavanagh K, Kitzman DW at al., Gut microbiome and aging: Physiological and mechanistic insights. Nutr Healthy Aging. 2018 Jun 15;4(4): 267-285.DOI: https://www.doi.org/10.3233/NHA-170030.
66. Vaiserman AM, Koliada AK, Marotta F. Gut microbiota: A player in aging and a target for anti-aging intervention. Ageing Res Rev. 2017 May; 35:36-45. DOI: https://www.doi.org/10.1016/j.arr.2017.01.001
67. Yadav H, Jain S, Nagpal R, Marotta F. Increased fecal viral content associated with obesity in mice. World J Diabetes. 2016 Aug 10;7(15):316-20. DOI: https://www.doi.org/10.4239/wjd.v7.i15.316.
68. Kumar M, Hemalatha R, Nagpal R, Singh B, Parasannanavar D, Verma V, Kumar A et al. Probiotic approaches for targeting inflammatory bowel disease: an update on advances and opportunities in managing the disease. Int J Probiotics Prebiotics. 2016;11(3-4):99-116.
69. Catanzaro R, Anzalone M, Calabrese F, Milazzo M, Capuana M, Italia A. et al. The gut microbiota and its correlations with the central nervous system disorders. Panminerva Med. 2015 Sep;57(3):127-43.
70. Mohania D, Kansal VK, Kumar M, Nagpal R, Yamashiro Y, Marotta F. Modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats. Acta Biomed. 2013 Sep 1;84(2):102-9.
71. Vasson MP, Farges MC, Goncalves-Mendes N, Talvas J, Ribalta J, Winklhofer-Roob B, Rock E, Rossary A. Does aging affect the immune status? A comparative analysis in 300 healthy volunteers from France, Austria and Spain. Immun Ageing. 2013 Sep 6;10(1):38. DOI: https://www.doi.org/10.1186/1742-4933-10-38.
72. Tylutka A., Morawin B., Gramacki A., Zembron-Lacny A. Pre-Existing Hypertension Is Related with Disproportions in T-Lymphocytes in Older Age. J Clin Med. 2022;11: 291. DOI: https://www.doi.org/10.3390/jcm11020291.
73. Zong J, Martirosyan DM.Anticancer effects of garlic and garlic-derived bioactive compounds and its potential status as functional food. Bioactive Compounds in Health and Disease. 2018; 1(2):16-35. DOI: https://www.doi.org/10.31989/bchd.v1i2.530
74. Haslberger A, Lilja S, Bäck H, Stoll C, Mayer A, Pointner A, Hippe B, Krammer U. Increased Sirtuin expression, senescence regulating miRNAs, mtDNA, and bifidobacteria correlate with wellbeing and skin appearance after Sirtuin- activating drink. Bioactive Compounds in Health and Disease. 2021; 4(4): 45-62. DOI: https://www.doi.org/10.31989/bchd.v4i4.801
75. Martirosyan DM, Ashoori MR, Rezaeinezhad A, Jahanbakhshi F, Pezeshki S, Mikaeili AS, Saham Alkhamis S et al. Effects of quercetin and low-level laser on oxidative and inflammatory factors among patients with type 2 diabetes mellitus and mild cognitive impairment. Bioactive Compounds in Health and Disease. 2022; 5(1): 1-12, DOI: https://www.doi.org/10.31989/bchd.v5i1.877
76. Cano M, Ayala A, Marotta F, Arguelles S. Application of Kinase Inhibitors for Anti-aging Intervention. Curr Pharm Des. 2017 Nov 16;23(29):4351-4368. DOI: https://www.doi.org/10.2174/1381612823666170714143426.
77. Muñoz MF, Argüelles S, Marotta F, Barbagallo M, Cano M, Ayala A. Effect of Age and Lipoperoxidation in Rat and Human Adipose Tissue-Derived Stem Cells. Oxid Med Cell Longev. 2020 Dec 9; 2020:6473279. DOI: https://www.doi.org/.10.1155/2020/6473279.
78. Vaiserman AM, Marotta F. Longevity-Promoting Pharmaceuticals: Is it a Time for Implementation? Trends Pharmacol Sci. 2016 May;37(5):331-333. DOI: https://www.doi.org/10.1016/j.tips.2016.02.003.
79. Sriramulu S, Banerjee A, Di Liddo R, Jothimani G, Gopinath M, Murugesan R, Marotta F, Pathak S. Concise Review on Clinical Applications of Conditioned Medium Derived from Human Umbilical Cord-Mesenchymal Stem Cells (UC-MSCs). Int J Hematol Oncol Stem Cell Res. 2018 Jul 1;12(3):230-234.