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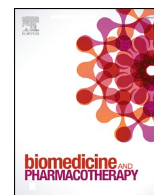

Propolis consumption by asymptomatic HIV-individuals: Better redox state? A prospective, randomized, double-blind, placebo-controlled trial

Karen Ingrid Tasca [a,*], Fernanda Lopes Conte [b], Camila Renata Correa [b], Karina Basso Santiago [a], Eliza de Oliveira Cardoso [a], Vanessa Martinez Manfio [b], Jessica Leite Garcia [b], Andresa Aparecida Berretta [c], Arthur Alves Sartori [a], Mariana da Silva Honorio [a], Lenice do Rosário Souza [b], José Maurício Sforcin [a]

[a] Institute of Biosciences, São Paulo State University (UNESP), Botucatu, SP, Brazil
[b] Botucatu Medical School, UNESP, Botucatu, SP, Brazil
[c] Apis Flora Industrial e Comercial Ltda., R&D Department, Ribeirão Preto, SP, Brazil

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ABSTRACT

Propolis is a natural product has many biological properties of clinical interest, such as anti-inflammatory and antioxidant. Considering that people living with HIV/aids (PLWHA) on effective combined antiretroviral therapy (cART) present early aging due to an intense immune activation, inflammation, and redox imbalance, propolis consumption could offer a benefit to such patients. This double-blind longitudinal study evaluated whether Brazilian green propolis pills intake (500 mg/day for three months) would decrease the oxidative stress of virological suppressed HIV-individuals. To compare each group (propolis, n = 20 versus placebo, n = 20) in both moments (M0, before and M1, after the intervention), the following markers were assessed: plasma malondialdehyde (MDA), carbonylation, total oxide nitric, total antioxidant capacity (TAP), superoxide dismutase, catalase, and NFkB and NRF2 gene expression. Data were analyzed using Poisson, Gamma distribution and ANOVA followed by Tukey-Kramer. The groups were homogeneous regarding age, gender, time of diagnosis/treatment, cART scheme, $CD4^+$ T cell count, and no changes were observed in the diet food, or patients' lifestyles. A decreased MDA concentration was seen in the propolis group (M0 = 0.24 ± 0.13, M1 = 0.20 ± 0.10 protein nmol/mg; $p = 0.005$) as well as a slight but non-significant increase of TAP (M0 = 49.07 ± 13.26, M1 = 52.27 ± 14.86%; $p = 0.06$). One may conclude that propolis promoted a lower lipid peroxidation and improved the antioxidant system, suggesting that its use may be beneficial to PLWHA in an attempt to contain the intense inflammatory and oxidant activity.

1. Introduction

The redox imbalance, which is constantly presented, is observed in several cohorts of HIV-infected individuals compared to non-infected individuals, with deficiency of the antioxidant protective system and high levels of oxidant products in various tissues [1–3]. Despite the better life expectancy and quality of life experienced by people living with HIV/aids (PLWHA) in the combined antiretroviral therapy (cART) era, they are affected by "early aging", which is a process characterized by the development of non-aids comorbidities (ex. diabetes, cardiovascular and metabolic diseases, etc) and immunosenescence at an earlier stage [4]. It is well documented that intense oxidative stress and chronic immune activation and inflammation contribute to this "early inflammaging" phenomenon [5].

The pathogenic role of oxidative stress in HIV disease was verified. This mechanism predicted all-cause mortality in HIV-infected patients. The same study showed that for plasma F2-isoprostanes, free radical-induced peroxidation products, this association was independent of

* Correspondence to: Lab. de Imunomodulação por Produtos Naturais. Instituto de Biociências de Botucatu (IBB-UNESP) - Departamento de Ciências Químicas e Biológicas. R. Dr. Plinio Pinto e Silva, S/N, Botucatu, SP CEP: 18618–691, Brazil.

E-mail addresses: karenitasca@hotmail.com, karen.i.tasca@unesp.br (K.I. Tasca), fer.conte@hotmail.com (F.L. Conte), correa.camila9@gmail.com (C.R. Correa), karina.basso@unesp.br (K.B. Santiago), eo.cardoso@unesp.br (E.O. Cardoso), vanessamanfio@hotmail.com (V.M. Manfio), jessleitegarcia@gmail.com (J.L. Garcia), andresa.berretta@apisflora.com.br (A.A. Berretta), arthursartorixd@gmail.com (A.A. Sartori), maashonorio@gmail.com (M.S. Honorio), lenice.souza@unesp.br (L.R. Souza), jose.m.sforcin@unesp.br (J.M. Sforcin).

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HIV-related variables and subclinical inflammation [6]. In a manner HIV itself is responsible for indirectly inhibiting the synthesis of some antioxidants [7] and its consumption is intensified by the pro-oxidant effect of inflammatory cytokines and/or activation of polymorphonuclear cells, which leads to an increased lipid peroxidation [8]. HIV *tat* protein is capable of inhibiting the transcription of the superoxide dismutase (SOD) gene, making the mitochondria vulnerable to reactive oxygen species (ROS) attacks which in turn activate nuclear factor kappa B (NFκB), increasing HIV transcription [9,10], and impairing DNA repair [11]. Thus, the cellular damage can also induce the reactivation of latent HIV, the progression of aids, and the presence of neoplasms and other non-aids comorbidities [1,2,11–13].

Despite significantly improving clinical, viral, and immunological parameters, cART seems to potentially contribute to this imbalance between oxidants and antioxidants, in synergy with HIV infection [1,3,13]. Since some of these drugs can activate the cytochrome C P450 enzyme system, they affect mitochondrial morphology and function [5,10] increasing the number of reactive species in the circulation, possibly due to increased production of oxidized metabolites derived from the interaction between ROS and biomolecules in infected cells [10]. Some antiretrovirals can either decrease the antioxidant protection or contribute to the failure of repair against oxidative damage. So, a significant number of patients with excellent adherence to cART had a higher oxidative status than non-adherent ones [1,13].

In this scenario, it is still necessary to find alternatives to improve the health of PLWHA to mitigate the impacts of the infection and the adverse effects of cART. Natural products may be the basis for developing new drugs to treat various diseases and infections, including HIV [14]. Hereupon, PLWHA could benefit from adjuvant therapies that include antioxidant strategies to overcome these deleterious effects [15].

Propolis is a bee product exerting a potent antioxidant property, eliminating ROS and activating cellular antioxidant systems [16]. Its composition depends on the geographical locations, plant sources or bee species [17] and includes resin, wax, balsam, essential oils, pollen, and plant primary and secondary metabolites (phenolics, amino acids, minerals, vitamins, terpenoids, tannins, and alkaloids) [18]. Propolis is one of the most abundant sources of polyphenols (mainly flavonoids and phenolic acids). It is known for its numerous therapeutic properties, highlighting its immunomodulatory, anti-inflammatory, antioxidant, antimicrobial and antiviral action [19].

Since HIV-infected people present high oxidative stress and chronic inflammation even during cART, we proposed to evaluate whether the intervention with propolis for three months could attenuate the oxidative stress of asymptomatic HIV-infected people with suppressed viral load.

2. Material and methods

2.1. Study design, participant eligibility and ethical issues

This prospective randomized double-blind placebo-controlled clinical trial enrolled 40 HIV-infected individuals from Specialized Outpatient Service for Infectious Diseases, “Domingos Alves Meira” (SAEI-DAM) - UNESP, Brazil.

To determine the sample size of the study, we adopted the variables “glucose, cholesterol, interleukins IL1-β and IL-10, and reactive oxygen species” reported by Al Ghamdi et al. [20]. A sample of 20 individuals/intervention per group was determined as necessary, assuming errors of type I and II (5% and 20%, respectively) and a loss of follow-up of 30%. This calculation was performed by professionals from the institution’s Research Support Office (EAP - FMB/UNESP).

Patients were randomly separated into two groups: propolis ($n = 20$) and placebo ($n = 20$). The random-numbers table (blocking randomization) was generated by an independent statistician. Participants and investigators were masked to group allocation.

Participants of propolis group ingested oral pills containing Brazilian green propolis – EPP-AF® 500 mg/day for 90 consecutive days [21]. Propolis-containing pills and placebo were kindly provided by the *Apis Flora Company*, Ribeirão Preto, SP, Brazil (Patent Letter nº 0405483–0, approved by Industrial Property Magazine on 23/07/2019). The chemical characterization and biological activities of the EPP-AF® standardized extract were previously reported by Berretta et al. [22] and Marquiafável et al. [23]. The placebo group received the pills without propolis.

The inclusion criteria were: HIV-seropositive asymptomatic patients; 20–55 years old; regular cART use for at least two years and good immunological response in the same period, verified by medication history system (for adherence) and the clinical exam history, i.e., undetectable plasma HIV-1 viremia levels (<40 copies of RNA/mL) and $CD4^{+}$ T lymphocyte count above 500 cells/mL for more than three years. In the inclusion period, there were no works in the literature discussing possible pharmacological interactions between propolis and antiretrovirals. Therefore, it was adopted a clinical stability of two years in the inclusion criteria to prevent viral rebound and to protect the participants.

Screened patients were excluded for any of the following criteria: pregnancy, use of illicit drugs or excessive alcohol consumption, cancer history, organ transplantation, chronic medical illness (cardiovascular diseases, diabetes mellitus, cancer, etc.) or co-infections (tuberculosis, chronic viral hepatitis, syphilis, human T-lymphotropic virus, papillomavirus and other sexually transmitted diseases), autoimmune or genetic diseases, intake of antibiotics, anxiolytics, antidepressants, vitamins, minerals, or other nutritional supplements.

This methodology was also proposed in previous studies conducted by our group, analyzing the same cohort. One of them reported the safety of propolis ingestion, detailed baseline characteristics, epidemiological, therapeutic, nutritional, and biochemical monitoring [24]. The consort flowchart of this clinical trial is also available in the related article [24]. All participants were instructed not to change their lifestyle/diet during the study and were accompanied by monthly phone calls.

This study was approved by the Research Ethics Committees of the Botucatu Medical School (FMB), UNESP (CAAE # 58694816.6.0000.5411) and by Brazilian Clinical Trials Registry (ReBec; # RBR-33mjbg).

2.2. Group characteristics and additional clinical analyses

The anthropometric and body composition assessment (bioimpedance) and dietary profile (24-hour dietary recall and self-reported food records) were registered for nutritional diagnosis. For sociodemographic and biochemical/metabolic analyses, it was taken the results of exams provided in the participants’ electronic records corresponding to the tests performed on the day of the blood collection for this study [24].

2.3. Redox biomarkers

For the analysis of plasma using fully validated procedures (see below), 12 mL of fresh blood samples were collected from each participant twice (M0, before and M1, after intervenction) by venepuncture into tubes containing anticoagulant EDTA. The whole blood was centrifuged at $500 \times g$ for 10 min, and the plasma aliquots were transferred into polypropylene tubes and stored at $-$ 80 °C until the performance of specific laboratory tests.

Total protein concentration was determined by a colorimetric method using the biuret reagent (*BioClin, Quibasa Química Básica Ltda., Belo Horizonte, Minas Gerais, Brazil – cat.n° K031*), and an automatic enzymatic analyser system (*Chemistry Analyzer BS-200, Mindray Medical International Ltd., Shenzhen, China*). The results were used to correct the redox state parameters.

A lipid peroxidation product (malondialdehyde [MDA])

concentration was determined as thiobarbituric acid reactive substances (TBARS) according to Buege and Aust [25]. Firstly, we prepared TBA reagent (15% TCA, 0.67% TBA, and 0.25 N HCl) to mixture 500 µl of this solution with 200 µl of plasma. After vortex, the mixture was centrifuged at 3500 rpm for 10 min (20 °C) and the pellet (300 µl) was removed with further heating for 45 min. After cooling, 200 µl of supernatant was plated and the absorbance was determined at 532 nm and at 600 nm for correction. Results were expressed as nmol MDA/mg protein using the molar extinction coefficient of chromophore (1.56 × 105 L/mol per cm).

Protein carbonylation –100 µl of plasma was incubated 10 min with 100 µ L of 2,4-dinitrophenylhydrazine (DNPH) (10 mM in 2 M HCl). It was added 50 µl of 6 M NaOH to the mixture and incubated for 10 min at room temperature. The absorbance was determined at 450 nm in a microplate reader and the result obtained from the absorbance of the samples and the molar extinction coefficient (22000 M-1 cm-1). Results were expressed as nmol of DNPH incorporated per milligram protein.

Total nitric oxide (NO) concentration was based on the enzymatic conversion of nitrate to nitrite by nitrate reductase by calorimetric reaction (Griess reaction) following the manufacturer's instructions (*R&D System - cat.n° KGE001*) using 50 µl of diluded plasma (1:4). Optical densities were expressed by µmol/L.

Total antioxidant capacity (TAP) was done using a VICTOR X2 reader (*Perkin Elmer-Boston, MA, USA*) [26]. This assay utilized 100 µl of plasma, which was incubated with the fluorescent indicator BODIPY (4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacene) for 10 min at 37 °C. Then the free radical generator 2,2'azobis (2-amidino-propane)-dihydrochloride (AAPH) was added to the samples. The prepared samples were applied in triplicates, 200 µl in each well on specific plates. Phosphatidylcholine (PC) was used as a hydrophilic matrix reference. The fluorescence reader (*Wallac Vitor 2X®, Perkin-Elmer, Boston, MA, USA*) was programmed to perform readings every 5 min for 3 h and 30 min. The results were reported as percentage of protection.

Antioxidant enzymes activity – Plasma was used to evaluate superoxide dismutase (SOD) and catalase (CAT) activities. SOD activity was measured based on the inhibition of a superoxide radical reaction with pyrogallol. This nitroblue-tetrazolic (NBT) reduction by free radicals is generated by hydroxylamine in alkaline medium (pH 10). Hydroxylamine generates O2- flow from NBT to blue-formazane at room temperature. When the sample is added, the rate of NBT reduction is inhibited according to the percentage of SOD present in the sample, and the absorbance values were measured at 420 nm [27]. CAT activity was evaluated in phosphate buffer pH 7.0 using 0.5 mL of sample by following the decrease in the levels of hydrogen peroxide (30%), and absorbance was measured at 240 nm [28]. The results were expressed as pmol/mg proteína/min (CAT) and U/mg proteína/min (SOD). The absorbance values for all analyses were measured in a UV/VIS spectrophotometer (*Pharmacia Biotech, Houston, Texas, USA*).

After plasma collection, the cells were centrifuged at 400 x g for 30 min at room temperature in *Ficoll-Paque* (*GE Healthcare Bio-Sciences, Sweden* – density = 1.077) to obtain the peripheral blood mononuclear cells (PBMC). The interface layer of PBMC was aspirated and washed twice with RPMI at 200 x g for 10 min

Gene expression of NF-kB and NRF2 transcription factors by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) - After PBMC separation, total RNA was extracted using the "Total RNA Purification" kit (*Norgen Biotek, Canada – cat.n° 37500*), according to the manufacturer's instruction. RNA concentration was evaluated spectrophotometrically using a NanoDrop 2000c (*Thermo Scientific, USA*), and its integrity was verified using the "Agilent RNA 6000 Nano Kit" (*Agilent Technologies, Germany – cat.n° 5067–1511*). Total RNA was incubated with RNase-free DNase (*Promega, USA*) to remove the genomic DNA. Subsequently, the synthesis of complementary DNA (cDNA) was performed, using ImProm-IITM Reverse Transcription System (*Promega, USA*). All gene expression was evaluated by RT-qPCR, using, for each well, 5 µl of GoTaq qPCR Master Mix (*Promega, Madison, WI, EUA*), 0.18 µl of Carboxy-X-Rhodamine (CXR) Reference Dye (identical to ROX™ dye; *Promega, Madison, WI, EUA*), 3.02 µl of ultrapure water / nuclease free (*Promega, Madison, WI, EUA*) and 0.4 µl of each primer (reverse and foward): NF-kB p65, RELa (NM_001145138.2): Forward - *CCCCTTCCAAGAAGAGCAGC* e Reverse - *TTGGGGGCACGATTGTCAAA*; NRF2 (NM_006164–5): Forward - *AACTACTCCCAGGTTGCCCA* e Reverse – *TGACTGAAACGTAGCCGAAG*; and GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase, NM_002046.4): Forward – *CGTGGAAGGACTCATGACCA* e Reverse – *GGCAGGGATGATGTTCTGGA*, used as an endogenous control to normalize gene expression. The reaction was carried out on 7300 fast real-time PCR Systems (*Applied Biosystems, USA*).

2.4. Statistical analyses

All analyses were performed with the assistance of professionals from the institution's Research Support Office (EAP - FMB/UNESP), using SAS for Windows (version 9.2).

Continuous variables were reported as means ± standard deviations, and the Shapiro-Wilk test was used to test their normality. In order to study the changes in both groups (propolis and placebo) over time (M0 and M1) and their interactions, a repeated measure design fitting a generalized linear model was adopted for continuous data: Poisson Distribution and Wald test for count variables; Gamma Distribution for asymmetric data; and Analysis of Variance (ANOVA) followed by Tukey's post-hoc test adjusted for variables under normal distribution. A *p value* of < 0.05 was considered to be statistically significant in this study.

3. Results

Both groups were homogeneous regarding gender (65.5% male in both groups), age (38.7 [± 7.9] years in placebo and 41.6 [± 7.2] in propolis group, p = 0.223), skin color (85% in placebo and 70% in propolis group were white, p = 0.106), time since HIV diagnosis (8.65 [± 5.25] years in placebo and 10.2 [± 5.91] in propolis group, p = 0.368) and cART duration (7.6 [± 4.37] years in placebo and 8.32 [± 5.27] in propolis group, p = 0.635). Additionally, the current $CD4^+$ and $CD8^+$ T lymphocyte count were similar between both groups ($p > 0.05$) with averages above 800 cells/m^3 (both CD4 + and CD8 + population) in both groups. Moreover, no differences were observed between the groups in the total cell blood count or biochemical variables, such as lipid profile, liver enzymes and kidney and liver function [24].

There was no difference in the diet, bioimpedance, or anthropometric measurements comparing the M0 and M1, i.e, the intake of macro and micronutrients was not changed from analysis of three food records made monthly, neither the body composition. All these results are showed in other study conducted by us [24].

In the present study, after 90-day propolis intervention, MDA concentration significantly decreased (M0 = 0.24 ± 0.13, M1 = 0.20 ± 0.10 nmol/mg protein; p = 0.005). Regarding TAP levels, only the propolis group tended to increase its protection percentage (M0 = 49.07 ± 13.26, M1 = 52.27 ± 14.86%; p = 0.06). There was no significant difference in carbonylation levels (Fig. 1).

There was a significant difference in SOD activity (M0 = 0.58 ± 0.03, M1 = 0.61 ± 0.05 pmol/mg protein; p = 0.033) only in the placebo group. Other markers values (NO, nitrite and catalase) were not significantly different in either of the groups before and after the intervention. Fig. 2.

Thus, we have extended this investigation by analyzing the expression of transcriptional factors NRF2 and NFκB, and surprisingly, an increased NFκB was seen after the intervention in both groups (Fig. 3).

4. Discussion

There are encouraging perspectives about propolis being promoted as a relevant protagonist position in medicine, not only in traditional,

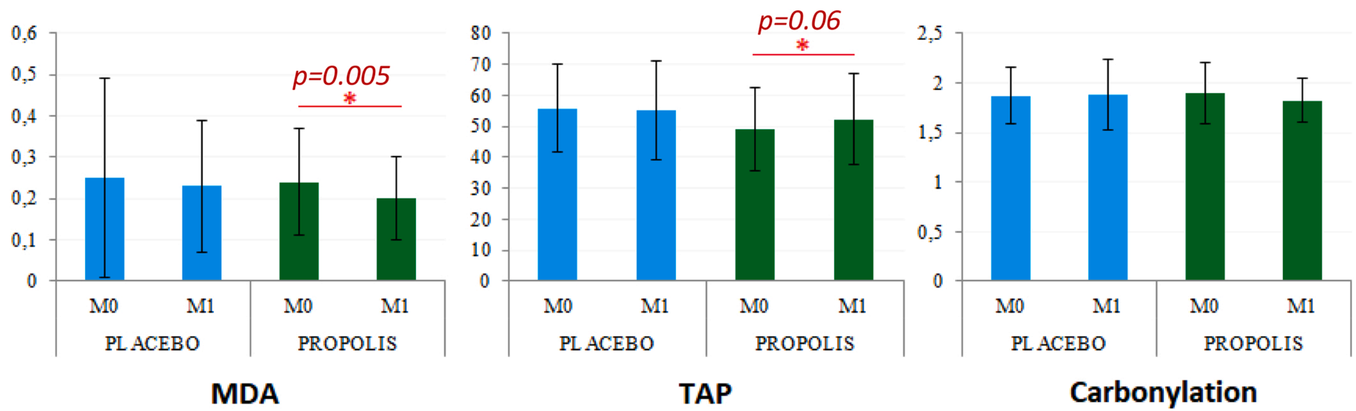

Fig. 1. Plasma lipid peroxidation product (malondialdehyde [MDA - nmol/mg protein]), total antioxidant capacity (TAP, % of protection) and protein peroxidation (carbonylation, nmol/mg protein) of 40 PLWHA, before (M0) and after (M1) the intervention (propolis vs placebo). Values represent means ± standard deviations. Statistical tests performed: Gamma distribution for MDA; Poisson distribution for TAP; Anova for carbonylation.

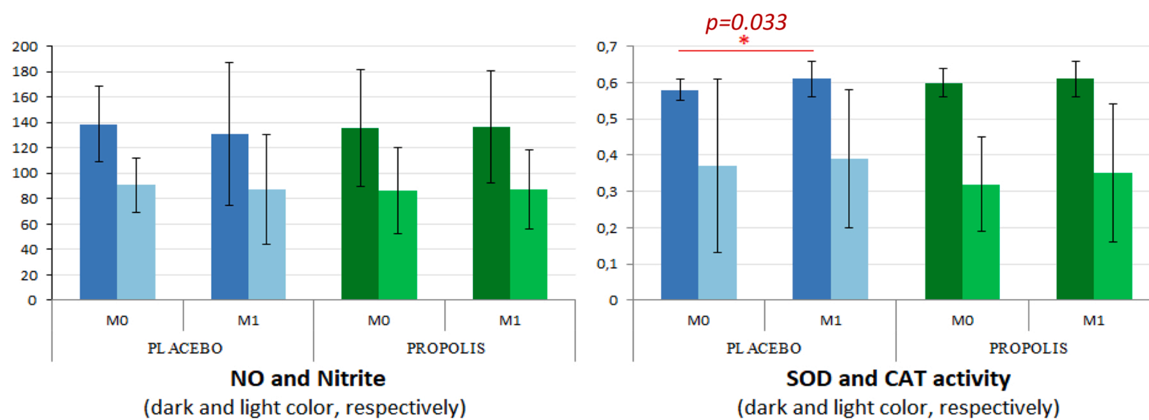

Fig. 2. Plasma oxide nitric (NO) and nitrite (μmol/L), and the activity of superoxide dismutase and catalase (SOD: U/mg proteína/min and CAT: pmol/mg proteína/min) of 40 PLWHA before (M0) and after (M1) the intervention (propolis vs placebo). Values represent means ± standard deviations. Statistical tests performed: Gamma distribution for CAT; Anova for SOD, NO and nitrite.

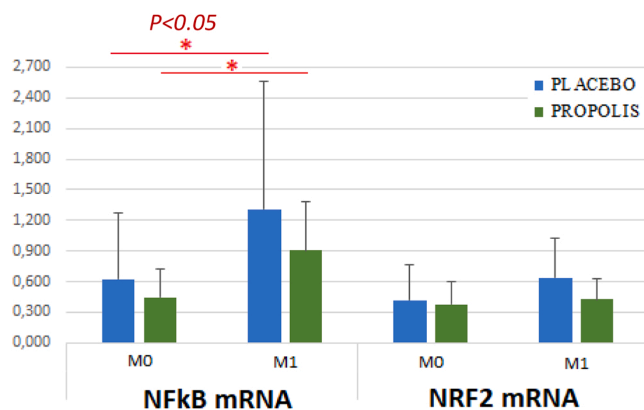

Fig. 3. Gene expression of NRF2 and NFκB by RT-qPCR (mRNA relative value) of 40 PLWHA before (M0) and after (M1) the intervention (propolis vs placebo). Values represent means ± standard deviations. Statistical test performed: Poisson distribution / Wald test.

complementary, and alternative medicine but also in clinical treatments [19]. In the field of HIV/aids, it is urgent to discover new natural compounds-based therapies that could potentially be more efficient than conventional cART, with fewer or no side effects [14], not to replace the effective treatment, but as an adjuvant to mitigate its side effects and improve the inflammatory/oxidative state, to consequently prevent the accelerated aging process commonly seen in PLWHA.

Brazilian propolis values a lot in the international market due to its beneficial effects on human health. As far as we know, there are only three controlled clinical studies in PLWHA that analyzed the effects of propolis: two conducted by our group, documenting the safety of its ingestion by this population - no adverse events or complaints during the study, no changes in the biochemical, metabolic, nutritional and dietary profiles except for the increment of magnesium [24]. We have also demonstrated an increased Foxp3 expression (Treg cells) and lymphocyte proliferation after propolis intervention. The other one was performed by Triyono et al. [29], who reported a gain of CD4 + T cells by the propolis group.

Here we proposed for the first time to investigate the influence of propolis supplementation in oxidative biomarkers in asymptomatic and virologically suppressed PLWHA.

In previous experimental studies, propolis contributed to a lower redox state. Usman et al. [30] evaluated its effect (300 mg/kg/day) on markers of placental oxidative stress in streptozotocin-induced diabetic rats. The rats treated with propolis and insulin, especially those using this combination, had a lower MDA and carbonylation, and a higher TAP, suggesting a protective effect of propolis in pregnant females with diabetes. Lisbona et al. [31] demonstrated that the consumption of propolis decreased the concentration of glucose and cholesterol, and reduced TBARS and protein oxidation in elderly rats, evidencing the antioxidant effect of this intervention in addressing the aging process.

Here, markers of lipid peroxidation, protein carbonylation, and

general antioxidant status were evaluated in the subjects, as well as total NO production after propolis intake. Neither the levels of protein carbonylation, nor NO concentration were altered after the intervention. There was a significant decrease in MDA ($p = 0.005$) and a slight improvement in TAP ($p = 0.066$) in the propolis-treated group, which suggested that propolis intake for 90 days could favor the reduction of oxidative stress in asymptomatic PLWHA on cART.

Other randomized double-blind studies in humans also pointed to this benefit. The study conducted by Afsharpour et al. [32], which supplemented patients' diets with 1500 mg of propolis daily, improved the antioxidant system (TAP and enzymes glutathione peroxidase [GPx] and superoxide dismutase [SOD]) of patients with type 2 diabetes. Mujica et al. [33] showed decreased lipid peroxidation and reduced glutathione (GSH) in healthy individuals after daily consumption of 15 drops of propolis for 90 days. Jasprica et al. [34] also noticed a decreased MDA and an increased SOD activity after 30 days of propolis use (48.75 mg of flavonoids per day for 30 days) in men but not in women. It is crucial to consider that most chronic diseases are associated with redox imbalance, including metabolic syndrome, atherogenicity, insulin resistance [35], renal compromising [36] and neurological diseases [37], and others. Therefore, our data and those from the literature lead to the hypothesis of multiple advantages in offering propolis to PLWHA, considering not only HIV infection but also the premature aging of this population.

Our promising results prompted us to discover if the increased TAP percentage could reflect a direct modulation in endogenous antioxidant pathways. However, propolis did not alter SOD or catalase activities, which contradicts other clinical studies. Diniz et al. [38], analyzing the same standardized extract used in the present study but at doses of 375 and 750 mg for 7 days, observed an increased SOD activity and a decreased lipid peroxidation in healthy people. In a randomized, double-blind, placebo-controlled study with diabetic patients supplemented with 1500 mg/day of propolis for eight weeks, Afsharpour et al. [32] showed an improvement in the total antioxidant system, including the enzymes SOD and glutathione peroxidase [GPx]. SOD was also higher in the study conducted by Jasprica et al. [34], evaluating men who consumed propolis (48.75 mg of flavonoids daily) for 30 days – but the same did not occur in women. Our findings may have been different for several reasons, including the characteristics of volunteers studied (health versus PLWHA on effective cART), the time of the intervention, dosage, the composition of the extracts and the study design. Yet, our HIV+ population has a reduced antioxidant capacity and pro-oxidant products increase due to the infection itself and the antiretroviral drugs administered [3,39].

Since propolis may contribute to attenuating some non-aids comorbidities, its phenolic and flavonoid components also seem to exert beneficial antihypertensive effects. According to Sun et al. [40], crude extract of Chinese propolis, as well as some of its isolated compounds, could be useful for the development of new anti-ischemic/myocardial drugs since in pre-treated H9c2 cardiomyocytes, there was an accumulation of SOD and GSH-Px in a dose-dependent manner after exposure to H_2O_2. Similarly, Zhou et al. [41] showed a reduction in the severity of hypertension induced by a diet rich in NaCl after administration of a water-soluble extract of propolis to rats. The endothelial protective effect occurred through several mechanisms of action, such as increased catalase activity and reduced vascular ROS levels [41]. ROS promote direct activation of the transcription factor NFκB, showing that oxidative stress contributes to an inflammatory state by inducing genes encoding pro-inflammatory cytokines, chemokines, leukocyte adhesion molecules, and other enzymes (cyclooxygenase, iNOS, etc) [42,43].

In this sense, it is relevant to evaluate factors that attenuate both inflammation and oxidative stress. The NRF2 pathway is responsible for ensuring cytoprotective effects, as its activation leads to an increase in the transcription of genes regulated by antioxidant response elements (ARE) that encode antioxidant enzymes [44]. The role of NRF2 in reducing inflammation is related to its ability to indirectly antagonize NFκB by removing ROS [43]. Thus, we proposed the investigation of NFκB and NRF2 mRNA expression to complement our analysis.

Propolis did not influence the gene expression of these transcription factors. There was an increased NFκB in both groups (propolis and placebo), with no change in the expression of NFR2. To maintain the balance of antioxidant and anti-inflammatory effects, it is possible that propolis activated the NRF2 pathway and inhibited the NFκB pathway [45]. It was documented that propolis or its isolated components could inhibit NFκB expression as mentioned by Conte et al. [46] investigating human monocytes using green propolis, by Wang et al. [47] and Abdel-Latif et al. [48], evaluating human lymphocytes and gastric epithelial cells treated with CAPE, by Paulino et al. [49] in several cell lines using artepilin C, and by Pahlavani et al. [50] in a review study.

Studies reported induction of NRF2 after propolis or its constituent administration with consequent suppression of ROS, and promotion in the transcription of several antioxidant enzymes, such as heme oxygenase-1, glutamate-cysteine ligase, GPx, GSH, SOD and catalase by its phenolic compounds and flavonoids [16,51–54]. However, all the aforementioned studies were performed in vitro using different stimuli and extracts/concentrations of propolis or its components, which differs greatly from our intervention in humans, whose gene analysis was performed directly from the PBMC of people who were on continuous propolis supplementation.

Other markers and pathways, which were not studied here, could contribute to the attenuation of the inflammatory and oxidative status promoted by propolis, since the anti-inflammatory properties of propolis may occur through the modulation of NFκB and mitogen-activated protein kinase (MAPK)MAPK [55,56]. Further studies are needed to optimize HIV infection management.

This study presents some limitations, such as reduced number of individuals included in each arm and the few markers used to represent the redox state of the participants. In addition, the absence of propolis influence on some markers studied here may have occurred probably due to the low dosage and short duration of the intervention established in our design, and these principles also deserve to be further explored. Nonetheless, our study had some strengths worth mentioning, such as its prospective, randomized, double-blind, placebo-controlled interventional character, the strict inclusion criteria adopted, the group homogeneity (sociodemographic, clinical and immunological variables), and the checked behavioral stability of participants regarding changes that could bias the study (such as possible changes in eating habits and lifestyle).

Finally, we indicated for the first time, the influence of propolis in improving the redox state of PLWHA. In this manner, propolis may provide several benefits - besides mitigating inflammation and oxidative damage, there is a potential to delay the onset of some underlying diseases that are more frequent, intense and appear earlier in this population.

5. Conclusion

The best redox status of the participants was verified 90 days after propolis intake, with a significant reduction in malondialdehyde concentration and an increased total antioxidant capacity / TAP. It is noteworthy that none of these differences were associated with changes in the eating habits of these individuals, nor with sociodemographic, clinical, and therapeutic characteristics.

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CRediT authorship contribution statement

KIT and **JMS** conceptualized and designed the research; **KIT** and **FLC** recruited and followed the participants; **CRC**, **AAB** and **LRS** provided reagents; **KIT, FLC, CRC, KBS, EOC, VMM, JLG, AAS** and **MSH** performed the laboratory experiments; **KIT, FLC, KBS** and **EOC** analyzed and interpreted the data; **KIT** wrote an early draft of the manuscript; **CRC, AAB, LRS** and **JMS** revised the manuscript. All authors have read and approved the final version of the paper.

Conflict of interest statement

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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References

[1] A. Mandas, E.L. Iorio, M.G. Congiu, C. Balestrieri, A. Mereu, D. Cau, et al., Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy, J. Biomed. Biotech. (2009) 749575–749577.

[2] B. Sharma, Oxidative stress in HIV patients receiving antiretroviral therapy, Curr. HIV Res. 12 (2014) 13–21.

[3] K.I. Tasca, J.T. Caleffi, C.R. Correa, M. Gatto, F.C. Tavares, C.C. Camargo, et al., Antiretroviral therapy initiation alters the redox system of asymptomatic HIV-infected individuals: a longitudinal study, Oxid. Med. Cell. Longev. 2017 (2017), 9834803.

[4] S. Pathai, H. Bajillan, A.L. Landay, K.P. High, Is HIV a model of accelerated or accentuated aging, J. Gerontol. A. Biol. Sci. Med. Sci. 69 (2014) 833–842, https://doi.org/10.1093/gerona/glt168.

[5] R.L. Smith, R. Boer, B. Stanley, Premature and accelerated aging: HIV or HAART? Front Genet. Genet. Aging 3 (2013) 328.

[6] M. Masiá, S. Padilla, M. Fernández, et al., Oxidative stress predicts all-cause mortality in HIV-infected patients, PLoS One 11 (2016), e0153456, https://doi.org/10.1371/journal.pone.0153456.

[7] J.A. Martin, J. Sastre, J.C. de la Asunción, F.V. Pallardó, J. Viña, Hepatic γ-cystathionase deficiency in patients with AIDS, J. Am. Med. Assoc. 285 (2001) 1444–1445.

[8] M.C. Delmas-Beauvieux, E. Peuchant, A. Couchouron, J. Constans, C. Sergeant, M. Simonoff, et al., The enzymatic anti-oxidant system in blood and glutathione status in HIVinfected patients: effects of supplementation with sele-nium or b-carotene, Am. J. Clin. Nutr. 64 (1996) 101–107.

[9] M.L. Gougeon, Apoptosis as an HIV strategy to escape immune attack, Nat. Rev. Immunol. 3 (2003) 392–404.

[10] T. Hulgan, J. Morrow, R.T. D'Aquila, S. Raffanti, M. Morgan, P. Rebeiro, et al., Oxidant stress is increased during treatment of human immunodeficiency virus infection, *Clin. Infect. Dis.* 37 (2003) 1711–1717.

[11] G. Nunnari, J.A. Smith, R. Daniel, HIV-1 Tat and AIDS-associated cancer: targeting the cellular anti-cancer barrier? J. Exp. Clin. Cancer Res. (2008) 27.

[12] D.R. Suresh, V. Annam, K. Pratibha, B.V. Prasad, et al., Total antioxidant capacity – a novel early bio-chemical marker of oxidative stress in HIV infected individuals, J. Biomed. Sci. 16 (2009) 1423.

[13] J.L. Ngondi, J. Oben, D.M. Forkah, L.H. Etame, D. Mbanya, The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon, AIDS Res. Ther. 3 (2006) 19.

[14] J. Popović-Djordjević, C. Quispe, R. Giordo, et al., Natural products and synthetic analogues against HIV: a perspective to develop new potential anti-HIV drugs, Eur. J. Med. Chem. 233 (2022), 114217, https://doi.org/10.1016/j.ejmech.2022.114217.

[15] C. Akay, M. Cooper, A. Odeleye, B.K. Jensen, M.G. White, F. Vassoler, et al., Antiretroviral drugs induce oxidative stress and neuronal damage in the central nervous system, J. Neurovirol. 20 (2014) 39–53.

[16] F. Zulhendri, M. Ravalia, K. Kripal, K. Chandrasekaran, J. Fearnley, C.O. Perera, Propolis in Metabolic Syndrome and Its Associated Chronic Diseases: A Narrative Review, Antioxid. (Basel) 10 (2021) 348.

[17] A. Salatino, M.L.F. Salatino, Scientific note: Often quoted, but not factual data about propolis composition, Apidologie 52 (2021) 312–314.

[18] S. Huang, C.P. Zhang, K. Wang, G.Q. Li, F.L. Hu, Recent advances in the chemical composition of propolis, Molecules 19 (2014) 19610–19632.

[19] A. Salatino, Perspectives for Uses of Propolis in Therapy against Infectious Diseases, Molecules 27 (2022) 4594.

[20] A.A. Al Ghamdi, G. Badr, W.N. Hozzein, A. Allam, N.S. Al-Waili, M.A. Al-Wadaan, et al., Oral supplementation of diabetic mice with propolis restores the proliferation capacity and chemotaxis of B and T lymphocytes towards CCL21 and CXCL12 by modulating the lipid profile, the pro-inflammatory cytokine levels and oxidative stress, BMC Immunol. 16 (2015) 54.

[21] A.A.Berretta, C. Arruda, F.G. Miguel, N. Baptista, A.P. Nascimento, F. Marquele-Oliveira, et al. Functional Properties of Brazilian Propolis: From Chemical Composition Until the Market. In: Superfood and Functional Food - AnOverview of Their Processing and Utilization. Waisundara VY, Shiomi N (editors). IntechOpen 2017, p. 55–98.

[22] A.A. Berretta, A.P. Nascimento, P.C. Bueno, M.M. Vaz, J.M. Marchetti, Propolis standardized extract (EPP-AF®), an innovative chemically and biologically reproducible pharmaceutical compound for treating wounds, Int. J. Biol. Sci. 8 (2012) 512–521, https://doi.org/10.7150/ijbs.3641.

[23] F.S. Marquiafável, A.P. Nascimento, H.S. Barud, F. Marquele-Oliveira, L.A. P. Freitas, J.K. Bastoset, et al., Development and characterization of a novel standardized propolis dry extract obtained by factorial design with high artepillin C content, J. Pharm. Technol. Drug Res. 4 (2015) 1, https://doi.org/10.7243/2050-120X-4-1.

[24] K.I. Tasca, F.L. Conte, A.C.M.M Alves, K.B. Santiago, E.O. Cardoso, L.B. Saciolotto, et al., Propolis intake by people living with HIV: biochemical profile, nutritional status and safety, Herbal Medicine. Submitted. (2023).

[25] J.A. Buege, S.D. Aust, Microsomal lipid peroxidation, Methods Enzym. 52 (1978) 302–310.

[26] G. Beretta, G. Aldini, R.M. Facino, R.M. Russell, N.I. Krinsky, K.J. Yeum, Total antioxidant performance: a validated fluorescence assay for the measurement of plasma oxidizability, Anal. Biochem. 354 (2006) 290–298, https://doi.org/10.1016/j.ab.2006.04.001.

[27] S.L. Marklund, Product of extracellular-superoxide dismutase catalysis, FEBS Lett. 184 (1985) 237–239.

[28] H. Aebi, Catalase in vitro, Methods Enzym. 105 (1984) 121–126.

[29] E.A. Triyono, S. Firdausa, H. Prasetyo, J. Susanto, J. Hutagalung, L. Masyfufah, et al., The effects of propolis extract administration on HIV patients receiving ARV, Indones. Biomed. 13 (2021) 75–83, https://doi.org/10.18585/inabj.v13i1.1381.

[30] U.Z. Usman, A.B.A. Bakar, M. Mohamed, Propolis improves pregnancy outcomes and placental oxidative stress status in streptozotocin-induced diabetic rats, BMC Complement. Altern. Med. 18 (2018) 324.

[31] C. Lisbona, J. Díaz-Castro, M.J. Alférez, I.M. Guisado, R. Guisado, I. López-Aliaga, Positive influence of a natural product as propolis on antioxidant status and lipid peroxidation in senescent rats, J. Physiol. Biochem. 69 (2013) 919–925.

[32] F. Afsharpour, S. Hashemipour, H. Khadem-Haghighian, Y. Koushan, Effects of Iranian propolis on glycemic status, inflammatory factors, and liver enzyme levels in type 2 diabetic patients: a randomized, double-blind, placebo-controlled, clinical trial, JNSD 3 (2017) 9–14.

[33] V. Mujica, R. Orrego, J. Pérez, P. Romero, P. Ovalle, J. Zúñiga-Hernández, M. Arredondo, E. Leiva, The role of propolis in oxidative stress and lipid metabolism: a randomized controlled trial, Evid. -Based Complement. Altern. Med. 2017 (2014) 11.

[34] I. Jasprica, A. Mornar, Z. Debeljak, A. Smolcić-Bubalo, M. Medić-Šarić, L. Mayer, et al., In vivo study of propolis supplementation effects on antioxidative status and red blood cells, J. Ethnopharmacol. 110 (2007) 548–554.

[35] N.R. Morelli, M. Maes, K.L. Bonifacio, H.O. Vargas, S.O.V. Nunes, D.S. Barbosa, Increased nitro-oxidative toxicity in association with metabolic syndrome, atherogenicity and insulin resistance in patients with affective disorders, J. Affect. Disord. 294 (2021) 410–419, https://doi.org/10.1016/j.jad.2021.07.057.

[36] P. Anvarifard, M. Anbari, A. Ostadrahimi, M. Ardalan, Z. Ghoreishi, A comprehensive insight into the molecular and cellular mechanisms of the effects of Propolis on preserving renal function: a systematic review, Nutr. Metab. 19 (2022) 6, https://doi.org/10.1186/s12986-021-00639-z.

[37] M.W. Park, H.W. Cha, J. Kim, et al., NOX4 promotes ferroptosis of astrocytes by oxidative stress-induced lipid peroxidation via the impairment of mitochondrial metabolism in Alzheimer's diseases, Redox Biol. 41 (2021), 101947, https://doi.org/10.1016/j.redox.2021.101947.

[38] D.P. Diniz, D.A. Lorencini, A.A. Berretta, M.A.C.T. Cintra, E.N. Lia, A.A. Jordão, E. B. Coelho, Antioxidant effect of standardized extract of propolis (EPP-AF®) in healthy volunteers: a "before and after" clinical study, Evid. -Based Complement. Altern. Med. 2020 (2020), 7538232.

[39] K.I. Tasca, C.R. Correa, J.T. Caleffi, M.B. Mendes, M. Gatto, V.M. Manfio, et al., Asymptomatic HIV People Present Different Profiles of sCD14, sRAGE, DNA Damage, and Vitamins, according to the Use of cART and CD4+ T Cell Restoration, J. Immunol. Res. (2018) 2018.

[40] L. Sun, K. Wang, X. Xu, M. Ge, Y. Chen, F. Hu, Potential protective effects of bioactive constituents from chinese propolis against acute oxidative stress induced by hydrogen peroxide in cardiac H9c2 cells, Evid. -Based Complement. Altern. Med. (2017), 7074147.

[41] H. Zhou, H. Wang, N. Shi, F. Wu, Potential protective effects of the water-soluble chinese propolis on hypertension induced by high-salt intake, Clin. Transl. Sci. 13 (5) (2020) 907–915.

[42] L.A. O'Neill, C. Kaltschmidt, NF-kappa B: a crucial transcriptionion factor for glial and neuronal cell function, Trends Neurosci. 20 (6) (2007) 252–258.

[43] J.E. Barbosa, M.B. Stockler-Pinto, B.O. Cruz, A.C.T. Silva, J.S. Anjos, C.T. Mesquita, et al., Perfil da Expressão do mRNA do Nrf2, NF-κB e PPARβ/δ em Pacientes com Doença Arterial Coronariana, Arq. Bras. De. Cardiol. 113 (6) (2019) 1121–1127.

[44] M.L. Pall, S. Levine, Nrf2, a Master Regul. Detoxif. also Antioxid., anti-Inflamm. Other Cytoprot. Mech., Is. Rais. Health Promot. Factors 67 (1) (2015) 1–18.

[45] W. Xu, H. Lu, Y. Yuan, Z. Deng, L. Zheng, H. Li, The antioxidant and anti-inflammatory effects of flavonoids from propolis via Nrf2 and NF-κB pathways, Foods 11 (16) (2022) 2439, https://doi.org/10.3390/foods11162439.

[46] F.L. Conte, K.I. Tasca, K.B. Santiago, E.O. Cardoso, G.G. Romagnoli, M.A. Golim, et al., Propolis increases Foxp3 expression and lymphocyte proliferation in HIV-Infected people: a randomized, double blind, parallel-group and placebo-controlled study, Biomed. Pharmacother. 142 (2021), 111984, https://doi.org/10.1016/j.biopha.2021.111984.

[47] L.C. Wang, K.H. Chu, Y.C. Liang, Y.L. Lin, B.L. Chiang, Caffeic acid phenethyl ester inhibits nuclear factor-kappaB and protein kinase B signalling pathways and induces caspase-3 expression in primary human CD4+ T cells, Clin. Exp. Immunol. 160 (2) (2010) 223–232.

[48] M.M. Abdel-Latif, H.J. Windle, B.S. Homasany, K. Sabra, D. Kelleher, Caffeic acid phenethyl ester modulates helicobacter pylori-induced nuclear factor-kappa B and activator protein-1 expression in gastric epithelial cells, Br. J. Pharm. 146 (8) (2005) 1139–1147.

[49] N. Paulino, S.R. Abreu, Y. Uto, D. Koyama, H. Nagasawa, H. Hori, et al., Anti-inflammatory effects of a bioavailable compound, artepillin C, in Brazilian propolis, Eur. J. Pharmacol. 587 (2008) 296–301.

[50] N. Pahlavani, M. Malekahmadi, S. Firouzi, et al., Molecular and cellular mechanisms of the effects of Propolis in inflammation, oxidative stress and glycemic control in chronic diseases, Nutr. Metab. (Lond.). 17 (2020) 65.

[51] H. Ichikawa, K. Satoh, T. Tobe, I. Yasuda, F. Ushio, K. Matsumoto, K. Endo, C. Ookubo, Free radical scavenging activity of propolis, Redox Rep. 7 (5) (2002) 347–350.

[52] A.L. Levonen, M. Inkala, T. Heikura, S. Jauhiainen, H.K. Jyrkkänen, E. Kansanen, et al., Nrf2 gene transfer induces antioxidant enzymes and suppresses smooth muscle cell growth in vitro and reduces oxidative stress in rabbit aorta in vivo, *Arterioscler. Thromb. Vasc.* Biol. 27 (4) (2007) 741–747.

[53] Y. Lee, D.H. Shin, J.H. Kim, S. Hong, D. Choi, Y.J. Kim, M.K. Kwak, Y. Jung, Caffeic acid phenethyl ester-mediated Nrf2 activation and IkappaB kinase inhibition are involved in NFkappaB inhibitory effect: structural analysis for NFkappaB inhibition, Eur. J. Pharmacol. 15 (1) (2010) 21–28, 643.

[54] S. Hotta, S. Uchiyama, K. Ichihara, Brazilian red propolis extract enhances expression of antioxidant enzyme genes in vitro and in vivo, Biosci. Biotechnol. Biochem 84 (2020) 1820–1830.

[55] M.S. Cho, W.S. Park, W. Jung, Z. Qian, D.S. Lee, J. Choi, et al., Caffeic acid phenethyl ester promotes anti-inflammatory effects by inhibiting MAPK and NF-κB signaling in activated HMC-1 human mast cells, Pharm. Biol. 52 (7) (2014) 926–932.

[56] B. Bueno-Silva, D. Kawamoto, E.S. Ando-Suguimoto, et al., Brazilian red propolis effects on peritoneal macrophage activity: nitric oxide, cell viability, pro-inflammatory cytokines and gene expression, J. Ethnopharmacol. 207 (2017) 100–107, https://doi.org/10.1016/j.jep.2017.06.015.