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Effects of propolis on inflammation markers in patients undergoing hemodialysis: A randomized, double-blind controlled clinical trial

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ABSTRACT

Background and aims: Several studies have been performed *in vitro* and in animals showing that propolis (a resin made by bees) has excellent anti-inflammatory properties, but no study has been performed in patients with chronic kidney disease (CKD) on hemodialysis (HD). The present study aimed to evaluate the effects of propolis supplementation on inflammatory markers in patients with CKD on HD.

Methods: This is a longitudinal, double-blind, placebo-controlled trial with patients randomized into two groups: propolis (4 capsules of 100 mg/day containing concentrated and standardized dry EPP-AF® green propolis extract) or placebo (4 capsules of 100 mg/day containing microcrystalline cellulose, magnesium stearate and colloidal silicon dioxide) for two months. Routine parameters were analyzed using commercial kits. The plasma levels of inflammatory cytokines were evaluated by flow luminometry.

Results: Forty-one patients completed the follow-up, 21 patients in the propolis group (45 ± 12 years, 13 women, BMI, 22.8 ± 3.7 kg/m^2) and 20 in the placebo group (45.5 ± 14 years, 13 women, BMI, 24.8 ± 6.8 kg/m^2). The obtained data revealed that the intervention with propolis significantly reduced the serum levels of tumour necrosis factor α (TNFα) ($p = 0.009$) as well as had the tendency to reduce the levels of macrophage inflammatory protein-1β (MIP-1β) ($p = 0.07$). There were no significant differences in the placebo group.

Conclusion: Short-term EPP-AF® propolis dry extract 400 mg/day supplementation seems to mitigate inflammation, reducing the plasma levels of TNFα and MIP-1β in patients with CKD on HD. This study was registered at clinicaltrials.gov (NCT04411758).

1. Introduction

Despite recent advances in dialysis solute and membrane technology, patients with chronic kidney disease (CKD) undergoing hemodialysis (HD) have several risk factors for cardiovascular diseases (CVD), which is the leading cause of mortality in these patients [1,2]. In addition to traditional risk factors (obesity, hypertension, diabetes, dyslipidemia) and typical situations of kidney disease (such as hypervolemia, anaemia and changes in calcium-phosphorus metabolism), patients on renal replacement therapy also have a high prevalence of emerging factors,

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such as oxidative stress and inflammation [2–5].

These patients have a high production of reactive oxygen species (ROS) and a reduction in the functioning of the body's antioxidant system. Oxidative stress is directly linked to inflammation since ROS promote the activation of nuclear factor κB (NF-κB), a transcription factor responsible for the synthesis of inflammatory cytokines such as interleukins IL-1, IL-6, IL8, IL-1β, IL-18, tumour necrosis factor α (TNF-α), interferon-γ (INF-γ) and other molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), which are closely associated with the progression of CVD, including acute myocardial infarction and congestive heart failure [6,7]. ROS also reduce the antioxidant capacity, caused mainly by impaired activation of factor 2-related erythroid nuclear factor 2 (Nrf2), one of the main cellular defense mechanisms against oxidative stress [8,9].

Therapeutic approaches to mitigate inflammation and oxidative stress are studied in patients with CKD, and nutritional interventions using food as medicine have been adopted for these patients [10]. Propolis, a resinous substance produced by bees from different plant exudates, has been used since ancient times for medicinal purposes and, currently, research has confirmed its biological effects, including anticancer, antidiabetic, antibacterial, antioxidant, and anti-inflammatory activities reducing the production of pro-inflammatory cytokines [11–14].

It is important to know that the chemical composition of propolis depends on the flora of the region where it comes from [15,16]. So, the propolis substances have a variable composition. The combination of its various chemical components determines its broad biological activity, such as pinocembrin, acacetin, chrysin, rutin, luteolin, kaempferol, apigenin, myricetin, catechin, naringenin, galangin and quercetin, caffeic, cinnamic and ferulic acids, prenylated phenylpropanids, and several others compounds including vitamins and minerals [11,12,17].

Some standardized extracts were already proposed offer batch-to-batch reproducible chemical profiles, such as Brazilian propolis standardized extracts EPP-AF® [18], which was already studied under the safety and efficacy point of view in several models, including human clinical trials [17,19–24].

There are no studies on the role of propolis in patients with CKD on HD. Only one study performed in non-dialysis CKD patients showed that supplementation of 500 mg/day of green propolis extract for 12 months reduced urinary MCP-1 (monocyte chemoattractant protein-1) and proteinuria in these patients [21]. Considering the vital safety and efficacy background of propolis extract, it could be proposed as an option to be studied focused on the benefit of patients with CKD and reducing its inflammatory process.

Thus, the possible effects of propolis in patients with CKD were discussed in a narrative review. It was concluded that this resin made by bees could attenuate inflammation in these patients [25]. Also, a recent systematic review showed that propolis might reduce oxidative stress and renal damage, including inflammation status [26].

The hypothesis proposed in this study is that propolis supplementation could reduce the inflammatory markers in patients with CKD undergoing HD. Therefore, this study aimed to evaluate the effect of propolis dry extract supplementation on inflammation in patients undergoing HD and its safety.

2. Methods

2.1. Propolis extraction and analysis

The green propolis standardized extract was kindly provided by Apis Flora Co. (Ribeirão Preto, São Paulo, Brazil). Propolis glycolic extract (PGE) was obtained from EPP-AF® after evaporation of the ethanol portion and to achieve 30% w/v of dry matter. The analysis was previously described by Sousa et al. (2007) [27]. The propolis extracts were analyzed by HPLC [18]. Table 1 shows the chemical characteristics of the propolis extract used. The total flavonoid as quercetin and the total phenolic as gallic acid were analyzed according to the method described above [18].

Table 1
Chemical characterization of the standardized propolis extract (EPP-AF®) used in this study.

Compounds	Standardized propolis extract (mg/400 mg)
Quercetin	20.0
Gallic acid	26.2
Artepellin C	25.9

2.2. Patients

This randomized, double-blind, placebo-controlled clinical trial with 41 hemodialysis patients was carried out from January to March 2021. Sample size calculation was performed a priori using G-Power 3.1 software for comparison of means between two groups (differences between post-intervention and baseline collections for placebo and propolis groups) by T-test. We assumed statistical power of 80%, a two-tailed significance level of 5%, an effect size (differences between groups) of 1, and a sample ratio between groups of 1, resulting in the desired sample size of 17 individuals per group. The primary endpoint was the TNF-α serum level.

The research was carried out after each participant signed the free and informed consent form, under the norms of the local Research Ethics Committees, based on the guidelines of the Declaration of Helsinki and the World Medical Association on research with human subjects. This study has been approved by the local ethics committee, and it is registered with ClinicalTrials.gov (NCT04411758).

2.3. Inclusion and exclusion criteria

Men and women with CKD on HD for at least six months, aged between 18 and 75 years, arteriovenous fistula (AVF) as vascular access, and patients with individualized dietary prescription (adequate energy supply 25–35 Kcal/ideal Kg/day and protein from 1.0 to 1.2 g/ideal Kg/day according to recommended by NKF-KDOQI, 2020) were included in the study. The HD sessions ranged from 3 to 4.5 h and occurred 3x/week, with a dialysate flow of 500 mL/min and a blood flow greater than 250 mL/min. Patients with autoimmune and infectious diseases, diabetes, cancer, and AIDS; pregnant, those using catabolic drugs, antibiotics, or antioxidant vitamin supplements (except for vitamin C, which is commonly used in the clinic), with regular intake of propolis and those who report being allergic to a bee sting, were excluded. Patients who had Covid-19 during the intervention period were also excluded.

2.4. Study design

In this clinical trial, patients were divided into two groups, the propolis group, in which CKD patients on HD received capsules containing standardized, concentrated and dry extract of dry green propolis and the placebo group, in which the patients received capsules containing microcrystalline cellulose, magnesium stearate and colloidal silicon dioxide. All the pots with the capsules were coded as A or B since it was a double-blind trial.

The dosage was two capsules of 100 mg after lunch and two after dinner for two months, totaling 400 mg per day of propolis.

The randomization of the groups was performed by an external person after checking the inclusion and exclusion criteria. Eligible patients were ranked 1:1 according to a computer-generated list of treatment codes. Blood tests, assessment of dietary intake, and anthropometric measurements were performed before and after the supplementation period with green propolis extract or placebo.

Regarding the adherence to the propolis supplementation, our staff verified through telephone contact, message by text, or by the app

WhatsApp® whether patients were taking the capsules as directed. At the end of the two months of supplementation, we calculated the remaining capsules in the bottles. With the difference between the capsules offered for two months of treatment and the capsules left over at the end, it was possible to establish the percentage of adherence of each patient by multiplying the number of capsules remaining by 100.

The assessment of nutritional status was carried out from the collection of anthropometric data obtained from the patient's medical records to minimize direct contact with patients as a result of the Covid-19 pandemic. It was used the dry weight (post HD) of the day on which the patients' blood samples were collected, and the same was used to calculate the BMI. Information on body weight and height was collected from the patient's medical records. A 3-day food recall assessed food intake.

2.5. Primary and secondary outcomes

The primary outcome was to evaluate the anti-inflammatory effects of propolis, and the secondary outcome was the safety of propolis evaluated by biochemical parameters and also by an adverse events questionnaire used in the study of Silveira et al. (2019) [21].

2.6. Collection and analysis of biological material

Blood samples were obtained in the morning from the arterial side of the hemodialysis access prior to the dialysis session. Blood was collected in Vacutainer® tubes containing ethylenediaminetetraacetic acid (EDTA) as the anticoagulant (1.0 mg/mL). To obtain the plasma and serum, the blood was centrifuged at 2500 rpm for 10 min at 4 °C and then stored at −80 °C until further analysis.

2.7. Multiplex test (serum) – inflammation markers

According to the manufacturer's recommendation, a multiplex bead-based assay was performed by the commercial kit and apparatus Bio-Plex Magpix (Biorad Laboratories Inc., Hercules, California, USA). The xMAP magnetic technology is based on microspheres that allow the detection of several different circulating proteins in the sample. In this study, serum levels of the following cytokines were analyzed: monocyte chemotactic protein 1 (MCP-1/CCL-2); macrophage inflammatory protein-1β (MIP-1β/CCL-4); interleukin-2, -6, -7, -8 (CXCL8), −10 e −17; tumor necrosis factor α (TNF-α).

2.8. Routine parameters

Plasma levels of biochemical parameters (albumin, glucose, total cholesterol, high-density lipoprotein (HDL), triglycerides, phosphorus, potassium, parathyroid hormone, hematocrit, and haemoglobin were analyzed using commercial kits from BioClin®.

2.9. Statistical analysis

Non-parametric Mann–Whitney U tests were used to compare continuous numerical baseline demographic and clinical variables, whereas Fisher's exact tests were used for categorical variables. The cytokine plasma levels were log (base 10) transformed and used as outcome parameters. Multiple linear mixed-effects models were used to evaluate the time-intervention interactions, where patients were considered a random effect. The model's fixed systematic component was adjusted by confounding variables (i.e., time on HD and BMI). The mean and 95% confidence intervals were used, and the results were also presented graphically for the estimated mean marginal effects. The sample distributions of data are represented in box plots (strip plots in grey and the black horizontal bars represent the 95% confidence intervals of the expected mean marginal effects by the group). The whole other variables made in the multiple linear mixed models remained in equal proportions or their average values, and contrasts were constructed from these estimated mean marginal effects. The Tukey Honest Significant Difference (HSD) method was used to correct p-values by the number of comparisons. It was considered statically significant p values ≤ 0.05. R software version 4.1.1, packages 'lme4', 'emmeans', and their dependencies were used to perform the statistical analyses.

3. Results

Forty-two patients completed the two months of intervention described in the CONSORT flow diagram (Fig. 1). The general characteristics of the patients studied are shown in Table 2, and no difference was observed between the groups at the baseline. Only two patients presented BMI below 18.5 kg/m^2 and five presented above 30 kg/m^2.

Regarding medication, 100% of all patients used antihypertensive drugs. The classes of antihypertensive drugs in descending order were: angiotensin II receptor blockers (58%), beta-blockers (41%), diuretics (33%), vasodilators (33%), angiotensin-converting enzyme inhibitors (16%) and calcium channel blockers (8%). Regarding the food intake, patients presented a protein intake of 0.93 g/kg/day and 23.6 kcal/kg/day.

The baseline biochemical parameters are presented in Table 3, and there are no statically differences between the groups at baseline. According to albumin, 28% presented values below the recommendation (>3.8 g/dL). Around 50% presented hyperphosphatemia (>5.5 mg/dL), 33% had high potassium levels (>5.5 mg/dL) and all patients presented anaemia according to haemoglobin values. Also, there is no evidence of plasma level differences after two months of intervention in both groups (data not shown). The values of dialysis adequacy (Kt/V) were in the adequate average and did not change after interventions. Additionally, to the biochemical analysis, the safety of supplementation was guaranteed, as there were no reports of adverse events that compromised the health of patients according to the questionnaire applied.

Table 4 shows the cytokine levels (expressed in Log 10) before and after each intervention. Fig. 2 illustrates the cytokine plasma levels of both treatment groups, comparing before and after the intervention of each group. After two months of supplementation, the inflammation parameters showed a significant reduction in the TNF-α and a tendency to reduce the MIP-1β serum levels in the propolis group.

4. Discussion

In this study, we show that supplementation with 400 mg of propolis per day for two months reduced the serum levels of TNFα and MIP-1β. To our knowledge, this is the first study evaluating propolis's anti-inflammatory activities in patients undergoing hemodialysis.

The main components of propolis are flavonoids and phenolic acids such as galangin and pinocembrin, well-known for their antioxidant action, with the ability to scavenge free radicals (donating hydrogen ions), neutralizing their action on cell membrane peroxidation and concomitantly apoptosis [28–30].

In the case of green propolis, prenylated cinnamic acid derivatives such as artepellin C, baccharin and drupanin are the key biomarkers and also participate in the biological activities of this product [17,31,32]. Artepellin C was demonstrated to control inflammation by down-regulating the mediators as NF-kB and reducing the inflammation symptoms such as pain, oedema and redness [33,34]. Artepellin C also decreased the synthesis of TNFα, IL-1β, IL-6, IL-8, IFN-γ and other inflammatory cytokines [33–39], besides the increase of IL-10 [37].

Furthermore, the inhibitory effects of propolis on NF-kB expression have already been elucidated in the literature [26,40–42]. Pinocembrin binds at the site of action and prevents the nuclear translocation of NF-kB to the nucleus, thereby inhibiting its activation [43]. Other bioactive compounds found in propolis, like caffeic acid phenethyl ester (CAPE), have a direct role in the inhibition of mRNA expression of NF-kB, responsible for the synthesis of several pro-inflammatory

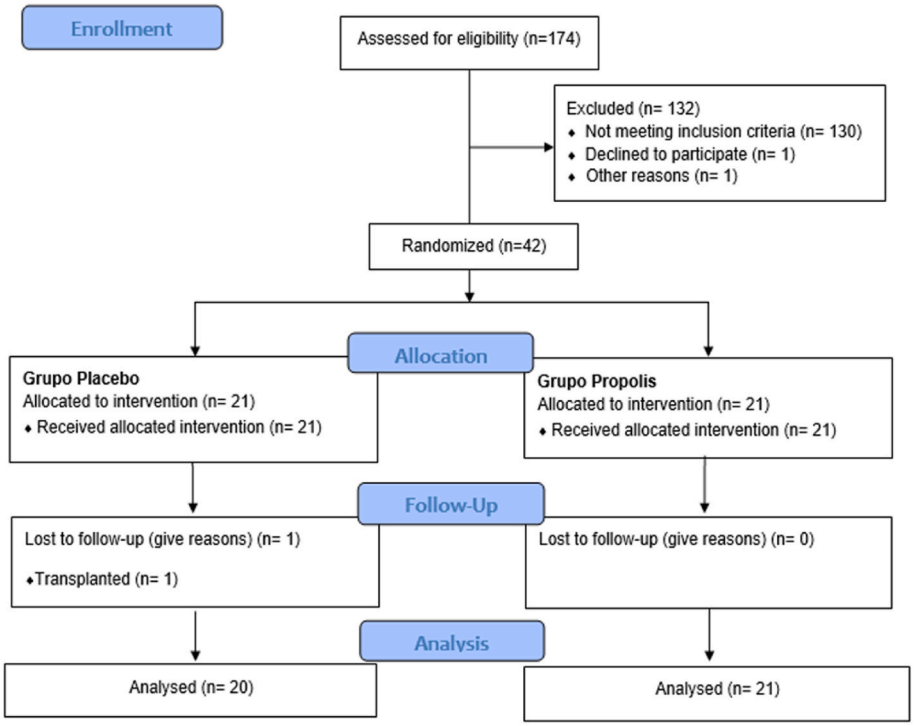

Fig. 1. CONSORT flow diagram of the study regarding propolis supplementation in patients with chronic kidney disease on hemodialysis.

Table 2
General characteristics between placebo and propolis groups at the baseline.

Parameters	Overall	Placebo	Propolis	p-value
Sex (F/M)	26/15	13/7	13/8	1.00
Age (yr)	45.0 (14.0)	45.5 (14.0)	45.0 (12.0)	0.85
Time on HD (months)	61.0 (70.0)	44.5 (46.5)	68.0 (60.0)	0.11
Kt/V	1.6 (0.5)	1.7 (0.5)	1.6 (0.4)	0.23
BMI (kg/m^2)	23.4 (4.7)	24.8 (6.8)	22.8 (3.7)	0.57

Data are presented as median (interquartile range – IQR) or absolute proportions.
Abbreviation: HD: hemodialysis; Kt/V – dialysis adequacy; BMI: body mass index.

Table 3
Biochemical parameters between placebo and propolis groups at the baseline.

Parameters	Overall	Placebo	Propolis	p-value
Total Cholesterol (mg/dL)	151.0 (35.5)	153.0 (40.5)	150.0 (25.2)	0.95
Albumin (mg/dL)	3.9 (0.3)	3.9 (0.2)	3.8 (0.3)	0.87
Phosphorus (mg/dL)	5.5 (2.4)	5.4 (2.7)	5.6 (1.5)	0.69
Glucose (mg/dL)	103.0 (27.0)	102.0 (22.5)	107.0 (29.0)	0.45
Potassium (mg/dL)	5.2 (0.6)	5.3 (0.6)	5.1 (0.8)	0.21
Haematocrit (%)	29.3 (3.7)	29.5 (5.6)	28.7 (5.1)	0.16
Haemoglobin (mg/dL)	9.5 (1.7)	9.4 (1.9)	9.6 (1.8)	0.67
Parathyroid hormone (pg/dL)	677.8 (857.5)	448.5 (541.9)	700.3 (800.4)	0.13

Data are presented as median (interquartile range – IQR).

Table 4
Plasma/Serum levels of cytokines (Log10) of placebo and propolis groups before and after each intervention.

Parameters (pg/mL)	Placebo		p-value	Propolis		p-value
	Pre	Pos		Pre	Pos	
IL6	0.39 (0.21; 0.57)	0.38 (0.13; 0.64)	1.00	0.15 (−0.02; 0.33)	−0.02 (−0.31; 0.27)	0.56
IL2	0.04 (0.03; 0.06)	0.03 (0.02; 0.04)	0.14	0.02 (0.01; 0.04)	0.02 (0.01; 0.03)	0.99
IL8	0.17 (0.07; 0.27)	0.21 (0.11; 0.31)	0.76	0.08 (−0.01; 0.17)	0.08 (−0.01; 0.17)	0.99
IL10	0.08 (−0.67; 0.84)	0.24 (−0.51; 1.00)	0.59	0.16 (−0.56; 0.89)	0.20 (−0.52; 0.92)	0.99
IL7	0.10 (0.05; 0.14)	0.06 (0.01; 0.11)	0.42	0.08 (0.04; 0.12)	0.08 (0.03; 0.12)	0.99
IL17	0.00 (0.00; 0.01)	0.00 (0.00; 0.01)	0.97	0.00 (0.00; 0.01)	0.00 (0.00; 0.01)	0.98
MCP1	0.25 (0.13; 0.37)	0.14 (0.01; 0.28)	0.48	0.19 (0.08; 0.30)	0.15 (0.04; 0.26)	0.87
TNF-α	0.76 (0.63; 0.90)	0.70 (0.57; 0.84)	0.17	0.58 (0.45; 0.70)	0.48 (0.35; 0.61)	0.00
MIP-1β	0.49 (0.43; 0.54)	0.44 (0.39; 0.49)	0.17	0.40 (0.35; 0.46)	0.35 (0.30; 0.40)	0.07

Data are presented as media (confidence range – IC).
Abbreviations: IL 6, IL 2, IL 8, IL 10, IL 7, IL 17: Interleukins 6,2,8,10,7,17; MCP1: Monocyte chemotactic protein 1; TNF-α: Tumor Necrosis Factor α; MIP1β: Macrophage inflammatory protein-1 beta.

cytokines with IL-1, IL-6, IL-8, IL-18, INF-γ, and TNFα [7,44,45]. Indeed, propolis can inhibit the synthesis of inflammatory markers such as C-reactive protein, TNFα, IL-6, and IL-1β [35].

Propolis efficiently reduced serum levels of TNFα by neutralizing ROS and activating the signaling pathway for its transcription in cutaneous anthrax animal models [46]. During the inflammatory process, TNFα also modulates the migration of neutrophils by stimulating endothelial cells to increase the expression of adhesion molecules, which are directly linked to adhesion, rolling, and transmigration of neutrophils [47]. Therefore, a possible way propolis modulates neutrophil

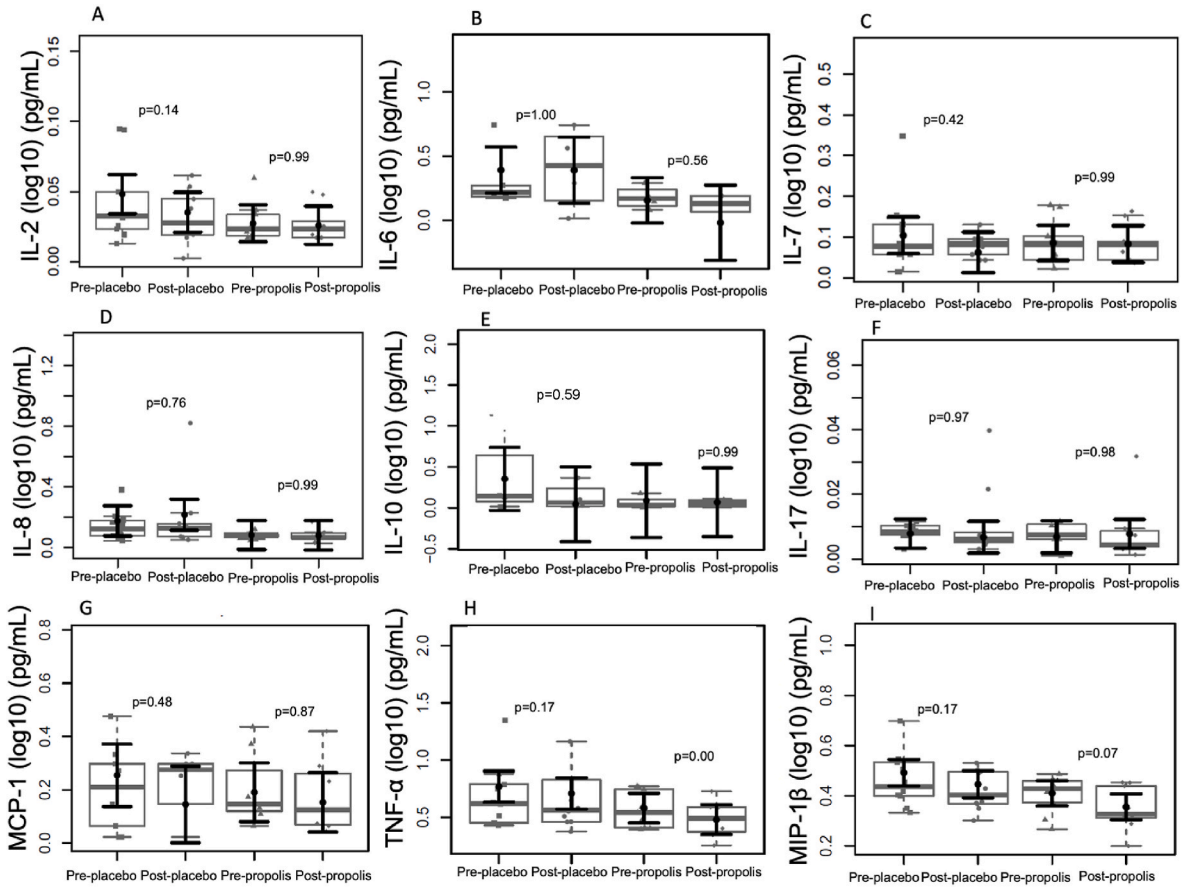

Fig. 2. Cytokines serum levels in both groups before and after each intervention. We found no evidence of interleukins serum levels differences after two months of intervention in both groups for interleukins-2, -6, -7, -8 (CXCL8), −10, −17 (**A - F**), nor for MCP-1 serum levels (**G**). However, we found significant differences for TNFα **(H)** and the tendency to reduce MIP-1β **(I)** after two months of intervention in the propolis group.

chemotaxis during the inflammatory process is through the inactivation of TNFα, which leads to a decrease in the activation and migration of neutrophils. It also acts via the CXCL2/MIP-2 chemokine, which, when activated, connects to its CXCR2 chemokine receptor and stimulates the influx of calcium into neutrophils, activating them. It was seen that propolis acts by blocking this influx of calcium and decreases the activation and migration of neutrophils [48].

MIP-1β is an important chemokine; among its functions, it is highlighted its role in the recruitment of leukocytes to the sites of infection, and in certain situations, it also acts by activating these cells [49]. In a study carried out in a cellular model, Szliszka et al. (2013) analyzed the action of Artepillin C isolated on macrophages, which suppressed the expression of MIP-1β and also inhibited the synthesis of several inflammatory cytokines. The same author also tested different concentrations of green propolis ethanol extract that also showed an inhibitory effect on MIP-1β, confirming the anti-inflammatory effects of propolis [50].

Still, on the immunomodulatory effect of propolis and its relationship with the inflammatory system, the study of Conte et al. (2021) performed in a cell model explains that propolis from southeastern Brazil reduced the TNFα concentrations by inhibiting the production of Th cytokines and reduced IL-6 concentrations by inhibiting the stimulation of retinoic acid in the production of this cytokine. In addition, propolis reduced NF-kB expression and increased IL-10 concentrations. These results are justified by propolis suppressing TLR-2 function (an important defense mechanism against pathogens) [41]. Machado et al. (2012) demonstrated that propolis increased IL-10 while reducing the inflammatory cytokines TNF-α and IL-6, proposing that the anti-inflammatory action is due to the immune regulation effects [37].

Furthermore, experimental and *in vitro* studies suggest that propolis restrains the production of leukotrienes, prostaglandins, and the enzyme prostaglandin-endoperoxide synthase and blocks COX-1 and COX-2 activation and regulates signaling pathways and modulation of B and T lymphocyte cell function [51–54].

As previously mentioned, galangin is another flavonoid present in propolis with an important anti-inflammatory function. Chaihongsa et al. (2021) showed that this flavonoid reduced blood pressure and improved the capacity of endothelium-related vasodilation in rats with induced hypertension. In addition, when analyzing the effects on the inflammatory system, suppression of TNF-R1, NF-kB, and VCAM-1 was seen in the aorta tissue, and TNFα serum levels reduced [55]. They also showed reduced damage caused by oxidative stress in a systemic way and in the vessel itself. This result was explained by the fact that galangin reduces the production of vascular O_2, thus having a lower reaction of O_2 with NO_2, which would produce more free radicals and consequently greater production of ROS [55].

Martin et al. (2021) observed a reduction in TNFα concentrations in patients with atopic dermatitis after the treatment with a cream containing green propolis extract (34–120 ng/mL) for 60 days [56].

Regarding the effects of propolis on oxidative stress, a study carried out with 67 subjects who had at least one altered parameter (fasting glycemia, lipids profile and blood pressure, or diabetes mellitus, cardiovascular disease, and/or overweight), where they received supplementation of 15 drops of propolis (3% solution preparation in propylene glycol) twice daily for 90 days showed a reduction in serum levels of TBARS, which was not seen in the placebo group [57].

A clinical trial with healthy volunteers showed that after seven days of using EPP-AF® (375 and 750 mg/day), the urinary excretion of 8-

isoprostanes (correlated with lipid peroxidation) and 8-OHDG (an important marker of oxidative DNA damage) was reduced [22].

Experimental studies (rats and rabbits) have shown that propolis supplementation reduces oxidative stress markers and increases the antioxidant enzymes [42,58].

Due to the coronavirus pandemic, much has been discussed about the role of propolis in this condition. In a recent clinical trial, Silveira et al. (2021) obtained clinically relevant results using propolis EPP-AF® extract at a dosage of 400 mg or 800 mg for seven days in hospitalized patients with COVID-19. The main results of this trial demonstrated shorter hospital stay in the supplemented groups, reduced administration of vasoactive drugs, and most importantly, a lower incidence of acute kidney injury, with the higher dose group having a significantly lower rate of acute kidney injury than the control group. Acute kidney injury is a frequent complication in patients with COVID-19 and is associated with a worse prognosis, extended hospital stays, and lethality [23]. The authors of this study did not specify the mechanism of action of propolis in COVID-19. However, in the review publication of Berretta et al. (2020), several proposals that justify the propolis effects on covid-19 patients are presented, as the activities of some biomarkers in this process [24]. In addition, another propolis component, kaempferol, inhibits the expression of TMPRSS2 and reduces the anchoring of ACE2, which the virus needs to invade host cells. All these actions are relevant in combating the evolution of patients infected with SARS-COV [23].

4.1. Limitations

It is known that propolis differs in composition according to each region of the country in which it is produced. Thus, one of the limitations is the region chosen in this study [15,16,32], where the number of total flavonoid compounds may have been lower than in the other studies used. However, advantageously green propolis possesses a prenylated group of compounds exclusively from *Baccharis dracunculifolia* exudates. Additionally, the propolis extract was standardized and patented to avoid the common variations observed with propolis. Another limitation to be considered is the supplementation time. Longer could be more effective. The third limitation is the dosage of propolis extract, and a high dose may promote a better anti-inflammatory effect. Regarding the safety of propolis, we cannot generalize these results; however, we showed that the dose and time of the propolis intervention were safe in this study.

5. Conclusions

Our results demonstrated that supplementation with propolis (400 mg/day) for two months reduced inflammatory cytokines such as TNFα and MIP-1β in patients with CKD on HD. However, the exact mechanism of action involved in regulating propolis and inflammatory pathway in these patients warrants further investigation.

Practical application

Nutritional interventions, including products rich in bioactive compounds such as propolis, have modulated inflammatory status in chronic diseases, including CKD. This study evaluated if propolis, a well-known possible resource to act in the immune system and inflammation in general, could also modulate inflammation and oxidative stress in hemodialysis patients. We showed that more studies are needed to evaluate such actions.

CRediT author statement

Borges NA and **Cardozo LFMF:** conceptualization, writing, **Leal VO:** conceptualization, methodology, writing, investigation; **Chermut TR, Fonseca LS** and **Figueiredo N:** writing, investigation, cytokines and other analysis, **Ribeiro-Alves M:** Statistical analysis, **Regis BP, Alvarenga L** and **Leite PEC:** cytokines and other analysis and writing, **Berretta AA** donating propolis capsules, writing, **Delgado A** writing, **Mafra, D:** conceptualization, funding acquisition writing, supervision, corrections.

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