Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Original article

Efficacy of Brazilian green propolis (EPP-AF®) as an adjunct treatment for hospitalized COVID-19 patients: A randomized, controlled clinical trial

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) promotes challenging immune and inflammatory phenomena. Though various therapeutic possibilities have been tested against coronavirus disease 2019 (COVID-19), the most adequate treatment has not yet been established. Propolis is a natural product with considerable evidence of immunoregulatory and anti-inflammatory activities, and experimental data point to potential against viral targets. We hypothesized that propolis can reduce the negative effects of COVID-19.
Methods: In a randomized, controlled, open-label, single-center trial, hospitalized adult COVID-19 patients were treated with a standardized green propolis extract (EPP-AF®) as an adjunct therapy. Patients were allocated to receive standard care plus an oral dose of 400 mg or 800 mg/day of green propolis for seven days, or standard care alone. Standard care included all necessary interventions, as determined by the attending physician. The primary end point was the time to clinical improvement, defined as the length of hospital stay or oxygen therapy dependency duration. Secondary outcomes included acute kidney injury and need for intensive care or vaso-active drugs. Patients were followed for 28 days after admission.
Results: We enrolled 124 patients; 40 were assigned to EPP-AF® 400 mg/day, 42 to EPP-AF® 800 mg/day, and 42 to the control group. The length of hospital stay post-intervention was shorter in both propolis groups than in the control group; lower dose, median 7 days versus 12 days (95% confidence interval [CI] −6.23 to −0.07; p = 0.049) and higher dose, median 6 days versus 12 days (95% CI −7.00 to −1.09; p = 0.009). Propolis did not significantly affect the need for oxygen supplementation. In the high dose propolis group, there was a lower rate

Abbreviations: ACE2, angiotensin-converting enzyme 2; CAPE, caffeic acid phenethyl ester; COVID-19, coronavirus disease 2019; NF-kB, nuclear factor-kappa B; PAK1, p21-activated kinase 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TLR4, toll like receptor 4; TMPRSS2, human transmembrane protease 2.

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of acute kidney injury than in the controls (4.8 vs 23.8%), (odds ratio [OR] 0.18; 95% CI 0.03–0.84; p = 0.048). No patient had propolis treatment discontinued due to adverse events.
Conclusions: Addition of propolis to the standard care procedures resulted in clinical benefits for the hospitalized COVID-19 patients, especially evidenced by a reduction in the length of hospital stay. Consequently, we conclude that propolis can reduce the impact of COVID-19.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic continues to cause considerable morbidity and mortality. More than 110 million people have been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) globally, resulting in over 2.4 million deaths [1] and unprecedented negative impacts on health care and the economy [2,3]. Despite advances in knowledge about viral targets for prospective medicines [4], COVID-19 remains a considerable therapeutic challenge [5]. Critical features of this disease that have been investigated for medicinal intervention include viral spike protein interaction with cellular angiotensin-converting enzyme 2 (ACE2) and the human transmembrane protease TMPRSS2, which allow SARS-CoV-2 to attach to and enter host cells [6,7]. In later stages of COVID-19, additional concerns include a typical exaggerated inflammatory response, mediated by p21-activated kinase 1 (PAK1), a "pathogenic" kinase [8–10], and the inflammasome [11], which are associated with pulmonary fibrosis, an increased need for intensive care and with high mortality rates [12]. Higher levels of PAK1 also reduce the adaptive immune response, facilitating viral replication [8,10,12]. Various propolis components can inhibit and/or modulate these viral targets [9,13].

Propolis, a natural product made by bees from bioactive plant parts and resins, is already extensively consumed in various regions of the world, due to its reputation as a health aid [9–16], including immunomodulatory properties [17,18] and antiviral activity [9,18–20]. It is classified as a conventional food or food supplement in Brazil, a dietary supplement in the USA, a food supplement in the European Union, a functional food in Japan and Korea, and a health food in China [15], being a low cost and easily accessible product.

Normally, propolis varies according to climate regions and to the types of plants available [15,16,21–23]. Differences between propolis products, due to a lack of standardization involving the botanical source, as well as differences in solvent extraction and processing methods, was a challenge identified by the European Medicine Agency, since it would be difficult to extrapolate the available safety and efficacy information for all types of propolis [14,16,18,24]. To overcome this problem with the variability of propolis, a standardized propolis product (EPP-AF®) that is chemically and biologically reproducible was developed [25,26]; it has proven safety and efficacy, and the dry extract had no significant interaction with medications, based on clinical studies conducted according to OMS guidelines [9,15,27,28]. Similar safety and efficacy were found in a clinical study using a poplar tree propolis extract [29], standardized according to recommendations made by Bankova [21].

1.1. Preclinical evidence

Preclinical studies have demonstrated the antiviral, anti-inflammatory, wound healing, anticancer, immunoregulating, neuroprotective, antiproteinuric and antioxidant properties of propolis [9,18,19,28,30–39]. Extensive revisions of the potential of propolis as a natural treatment option for COVID-19 [9,18,41–45] provide considerable evidence that it is an option that merits testing. Propolis components potentially could interfere with TMPRSS2 expression and ACE2 anchorage [16,46]. They also could help reduce inflammatory processes by PAK1 inhibition [8–10], and by inhibiting the inflammasome [31].

Given the evidence concerning its activities based on in vitro and in vivo research, we hypothesized that propolis could reduce the clinical impact of COVID-19 without interfering with other treatment options. To evaluate the efficacy and safety of oral propolis for SARS-CoV-2 infection, we conducted a randomized, controlled, open-label trial in Salvador, Bahia, Brazil: Bee-Covid (The Use of Brazilian Green Propolis Extract (EPP-AF®) in Patients Affected by COVID-19).

2. Materials and methods

2.1. Trial design and oversight

Bee-Covid was a single-center, open-label, randomized, controlled trial conducted from June 3 through August 30, 2020, at São Rafael Hospital, Salvador, Bahia, in northeast Brazil. Because of the emergency nature of the trial, placebos were not prepared.

The protocol was approved by the Brazilian Committee of Ethics in Human Research (Registration number 31099320.6.0000.0048), approved May 30, 2020, and the trial was registered (ClinicalTrials.gov number, NCT04480593). The study was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. All participating patients and/or legal representatives were informed about the objectives and risks of participation and gave written informed consent.

Eligible patients were randomly assigned in a 1:1:1 ratio to receive Propomax® capsules produced with dehydrated Standardized Brazilian Green Propolis Extract, EPP-AF® [25] for seven days at 400 mg/day (one 100 mg capsule, four times a day) plus standard care, or 800 mg/day (two 100 mg capsules, four times a day) plus standard care, or standard care alone (control group). The decisions on standard supportive treatment were made by the attending physicians, who were not involved in the study design or in the randomization process. Standard care comprised, as necessary, supplemental oxygen, noninvasive or invasive ventilation, corticosteroids, antibiotics and/or antiviral agents, vasopressor support, renal-replacement therapy, intra-aortic balloon pump and extracorporeal membrane oxygenation.

The dose of propolis was chosen based on studies that had used similar doses without observing adverse effects [15,27,28]. Patients were assessed daily during their hospitalization, from days 1 through 28. Data from patients who could not be reached for the 28-day follow-up were censored at hospital discharge. A standardized Brazilian green propolis extract, which is composed mainly of a green propolis produced in southeast Brazil, processed with a specific extraction and drying process, was selected for use in this study because of its batch-to-batch reproducibility [25,26]. The dosage of 400 mg/day offered 21.2 mg of total flavonoids, such as quercetin (measured according to procedures previously described by Woisky and Salatino [47]) and 54 mg of total phenolics, such as gallic acid (according to Rocha et al. [48]).

2.2. Randomization and masking

Randomization was stratified based on age, degree of pulmonary involvement, comorbidities, symptom onset time, and oxygen requirement as factors. The randomization scheme was generated by using the web site Randomization.com <http://www.randomization.com>. The randomization sequence was per block (using permuted blocks with four patients per block), including stratification. To minimize bias, allocation concealment was performed by researchers not involved in patient care. Maximum blindness among health professionals who had contact with

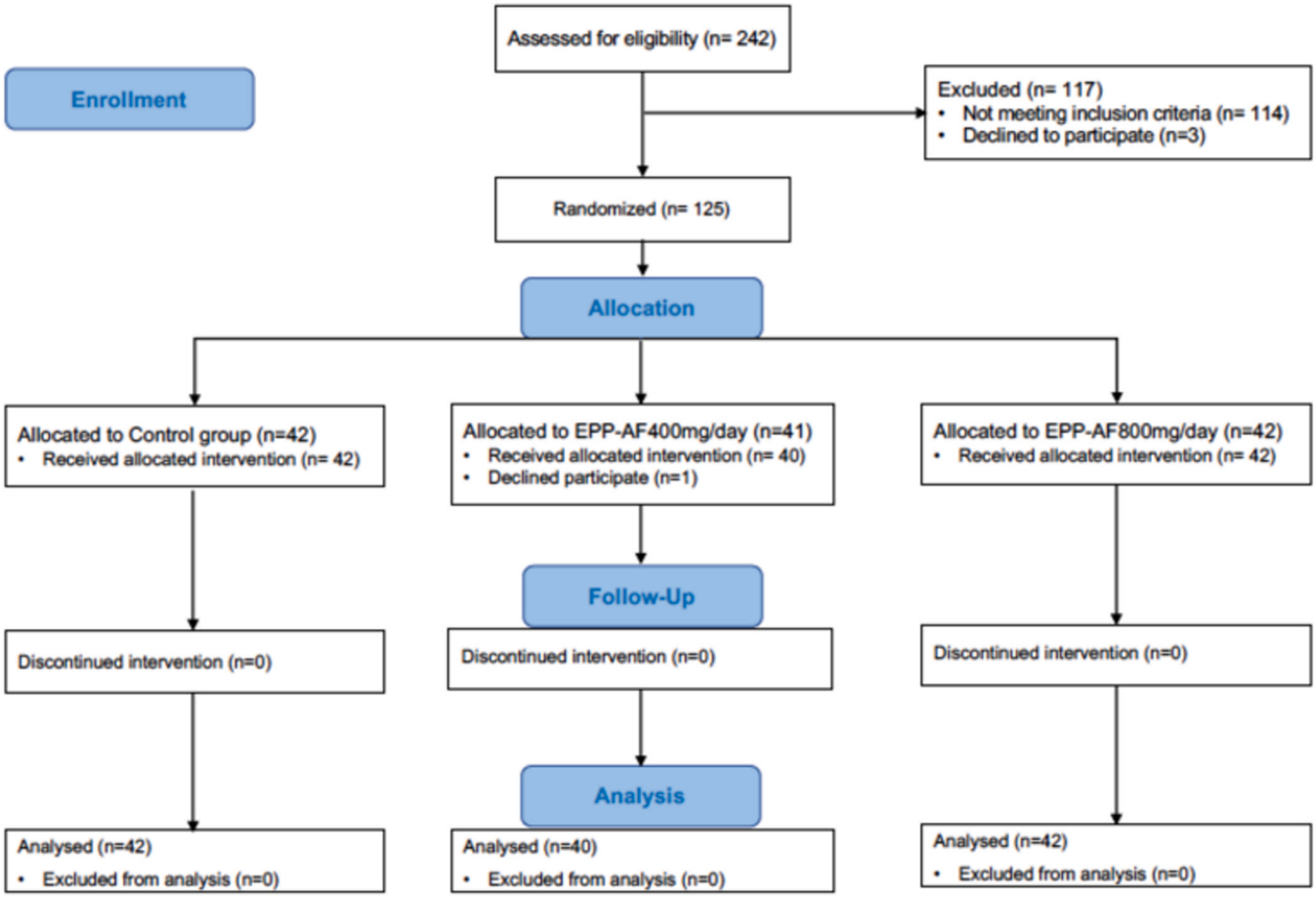

Fig. 1. Consort flow diagram for the BeeCovid study.

the participants was sought; the professionals responsible for caring for patients did not have access to the intervention proposed in this study, and the data analysis was carried out with external statistical support and in an impartial manner.

2.3. Patients

Hospitalized patients over 18 years of age diagnosed with SARS-CoV-2 infection, confirmed by polymerase chain reaction–reverse transcriptase testing, were considered eligible if symptoms started within 14 days of the randomization date. Exclusion criteria included pregnancy or lactation, known hypersensitivity to propolis, active cancer, human immunodeficiency virus carriers, patients undergoing transplantation of solid organs or bone marrow or who were using immunosuppressive medications, bacterial infection at randomization, sepsis or septic shock related to bacterial infection at randomization, impossibility of using the medication orally or by nasoenteral tube, known hepatic failure or advanced heart failure (New York Heart Association [NYHA] class III or IV).

2.4. Outcome measures

The primary end point was the time to clinical improvement defined as the length of hospital stay or oxygen therapy dependency time. Secondary end points were the percentage of participants requiring mechanical ventilation, rate of acute kidney injury, need for renal replacement therapy, need for intensive care treatment, and need for vasoactive drugs. We also analyzed patient laboratory parameters, including variation in serum levels of C-reactive protein over the seven days after randomization (Tables A1 and A2), and death.

Acute kidney injury was defined according to Kidney Disease: Improving Global Outcomes (KDIGO) criteria [49] as stage 1 (increase in serum creatinine by 0.3 mg/dl within 48 h or a 1.5–1.9 times increase in serum creatinine from baseline within 7 days), stage 2 (2.9 times increase in serum creatinine within seven days), or stage 3 (3 times or more increase in serum creatinine within seven days or initiation of renal replacement therapy.

Safety outcomes included adverse events that occurred during treatment, serious adverse events, and premature or temporary discontinuation of treatment. Adverse events were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.

2.5. Statistical analysis

As information about the use of propolis for respiratory syndrome conditions was limited, we used data from previous studies to infer the length of hospital stay due to COVID-19 [50]. We assumed a mean ($\pm$ standard deviation) length of hospital stay of 13 $\pm$ 6.5 days in the control group. Based on a two-sided type I error of 0.05 and 80% power to identify a difference of four days of length of hospital stay between the lower dose and the control groups, a sample size of 42 patients by group would be needed. This number of patients were recruited for the group with the higher propolis dose and the control group.

The primary analysis study population comprised all patients who had been randomized (intention-to-treat population), using the group to which a patient was allocated as a variable, regardless of the medication administered or treatment adhesion. The main objective was to evaluate the effectiveness of propolis in reducing the number days of oxygen therapy and length of hospital stay in adult patients with confirmed

Table 1
Demographic and clinical characteristics of the COVID-19 patients at baseline.

Variables	Total (N = 124)	Standard care (N = 42)	EPP-AF 400 mg/day (N = 40)	EPP-AF 800 mg/day (N = 42)
Age, mean (SD), y	50.0 (12.8)	51.6 (14.3)	49.5 (12.8)	48.9 (11.2)
Male sex, No. (%)	86 (69.4)	28 (66.7)	28 (70.0)	30 (71.4)
Ethnicity, No. (%)				
White	33 (30.6)	10 (23.8)	13 (32.5)	10 (23.8)
Black	26 (21.0)	12 (28.6)	5 (12.5)	9 (21.4)
Mixed	65 (52.4)	20 (47.6)	22 (55.0)	23 (54.8)
Coexisting conditions, No. (%)				
Diabetes	26 (21.0)	11 (26.2)	10 (25.0)	5 (11.9)
Hypertension	56 (45.2)	21 (50.0)	18 (45.0)	17 (40.5)
COPD/Asthma	9 (7.3)	2 (4.8)	3 (7.5)	4 (9.5)
Obesity	64 (51.6)	18 (42.9)	23 (57.5)	23 (54.8)
Median time from symptom onset to randomization, median (IQR)	8.0 (6.0–10.0)	8.0 (6.0–10.7)	8.0 (5.0–9.0)	9 (7.0–10.0)
Randomization location, No. (%)				
Ward	73 (58.9)	22 (52.4)	27 (67.5)	24 (57.1)
ICU	51 (41.1)	20 (47.6)	13 (32.5)	18 (42.9)
Conditions at randomization, No. (%)				
No additional oxygen therapy	60(48.4)	20 (47.6)	20(50.0)	20(47.6)
Nasal cannula	59(47.6)	21(50.0)	17 (42.5)	21 (50.0)
High-flow nasal cannula	1 (0.8)	0 (0.0)	1 (2.5)	0 (0.0)
Invasive ventilation	4 (3.2)	1 (2.4)	2 (5.0)	1 (2.4)
Temperature, median (IQR), °C	36.3 (35.7–36.8)	36.4 (35.9–36.8)	36.2 (35.7–36.8)	36.2 (35.5–37.0)
Respiratory rate >24/min — No. (%)	12 (9.7)	5 (11.9)	4 (10.0)	3 (7.1)
SpO2 < 93%, No. (%),	21 (16.9)	4 (9.5)	6 (15.0)	11 (26.2)
Lung parenchyma involvement estimated by CT, No. (%)				
<25%	29 (23.4)	10 (23.8)	13 (32.5)	6 (14.3)
25–50%	62 (50.0)	20 (47.6)	14 (35.0)	28 (66.7)
50–75%	29 (23.4)	11 (26.2)	10 (25.0)	8 (19.0)
>75%	4 (3.2)	1 (2.4)	3 (7.5)	0 (0.0)
Concomitant COVID-19 treatment[a], No. (%)				
Azithromycin	118 (95.2)	41 (97.6)	37 (92.5)	40 (95.2)
Chloroquine or Hydroxychloroquine	4 (3.2)	2 (4.8)	0 (0.0)	2 (4.8)
Oseltamivir	76 (61.3)	28 (66.7)	24 (60.0)	24 (57.1)
Corticosteroids	100 (80.6)	39 (92.9)	26 (65.0)	35 (83.3)
Creatinine (mg/dl), median (IQR)	0.81 (0.62–1.00)	0.68 (0.55–1.02)	079 (0.67–1.00)	0.85 (0.76–1.03)
White-cell count (x10^3/mm^3), median (IQR)	5.9 (4.3–7.9)	6.2 (4.4–7.8)	5.3 (3.6–7.1)	6.1 (4.8–8.2)
Lymphocyte count (x 10^3/ mm^3), median (IQR),	0.9 (0.7–1.3)	0.82 (0.7–1.0)	1.0 (0.7–1.4)	1.0 (0.7–1.3)
Platelet count (x 10^3/ mm^3), median (IQR)	183.0 (142.5–233.5)	164.0 (131.0–224.2)	201.5 (145.7–239.5)	188.5 (154.2–235.7)
Aspartate aminotransferase (U/L) — median (IQR)	43.0 (32.5–63.0)	46.5 (31.7–65.2)	49.0 (31.5–73.5)	39.0 (33.0–49.5)

Abbreviations: BMI, body-mass index (calculated as weight in kilograms divided by height in meters squared), COPD, chronic obstructive pulmonary disease, CT computerized tomography, SpO2, Peripheral oxygen saturation.

[a] lopinavir-ritonavir, remdesivir, tocilizumab, and colchicine were not used for any patient.

SARS-CoV-2 infection. The number of days on oxygen therapy was based on the number of days patients were on invasive or noninvasive oxygen therapy, both counted after randomization. The time to discharge or oxygen free was assessed after all patients had reached day 28. Patients still dependent on oxygen therapy, or still hospitalized, or who died by the end of follow-up time, were considered as time equal 28 days in the analysis. The endpoints were assessed visually using Kaplan Meier curves. The treatment effect was presented as the mean difference, with 95% confidence intervals and p-values. We used a generalized linear model with a Gamma distribution, considering age and the treatment groups as independent variables.

The binary outcomes were assessed with a logistic-regression model. Continuous outcomes were evaluated through linear regression. Ventilator time, intensive care unit time, and vasopressor drug use time were adjusted additionally for the status at randomization. Adverse events were expressed as counts and percentages and compared between groups using the Fisher exact test. Analyses were performed with R software, version 4.0.2 (R Project for Statistical Computing).

3. Results

3.1. Patients

Of the 242 patients who were assessed for eligibility, 125 met inclusion criteria, were enrolled, and underwent randomization and 124 began treatment: 40 patients were assigned to receive the lower dose of propolis (400 mg/day), 42 to receive the higher dose of propolis (800 mg/day) and 42 to receive standard care alone (control group) (Fig. 1). One patient was excluded after randomization and before receiving the medication (withdrew consent after randomization).

The mean (± standard deviation) age of patients in this trial was 50.0 ± 12.8 years, and 69.4% of the patients were men (Table 1). Overall, 5.2% of patients had hypertension, 51.6% were obese, 21.0% had diabetes, and 7.3% had chronic pulmonary obstructive disease. The median (interquartile range) time from symptom onset to randomization was 8 (6–10) days. At randomization, 3.2% were using invasive mechanical ventilation, 48.4% were receiving oxygen with non-invasive ventilation, and 41.1% were being treated in the intensive care unit.

Follow-up information at day 28 after admission for the primary outcome was complete for all 124 patients. The use of azithromycin, chloroquine, hydroxychloroquine, or oseltamivir was similar in all groups. The frequency of patients requiring use of corticosteroids was lower in the group receiving the lower dose of propolis (65%) when compared to the higher dose of propolis (83%) and standard care (93%) groups.

Table 2
BeeCovid study outcomes.

Outcomes	Control Group (n = 42)	EPP-AF 400 mg/day (n = 40)	EPP-AF 800 mg/day (n = 42)	Effect statistic	Between-group effect Adjusted[a]			
					Estimate EPP-AF 400 mg/day (95% CI)	*P* value	Estimate EPP-AF 800 mg/day (95% CI)	***P* value**
Primary outcome (Duration in days)								
Hospital stay								
Mean (95% CI)	12.6 (10.6–14.6)	9.5 (7.2–11.8)	8.2 (6.5–9.9)	MD	−3.03 (−6.23 to −0.07)	0.049	-3.88 (−7.00 to −1.09)	0.009
Median (IQR)	12 (8–16)	7.0 (5–12)	6.0 (5–11)					
Oxygen therapy[b]	(N = 33)	(N = 21)	(N = 27)					
Mean (95% CI)	7.4 (4.9–10.0)	6.3 (2.9–9.8)	5.0 (2.9–7.2)	MD	−2.13 (−7.84 to 3.57)	0.470	-0.99 (−6.09 to 4.12)	0.710
Median (IQR)	5 (3–11)	3 (1–6)	2 (1–5)					
Acute Kidney Injury, No. (%)	10 (23.8)	5 (12.5)	2 (4.8)	OR	0.51 (0.13–1.80)	0.305	0.18 (0.03–0.84)	0.048
AKI KDIGO 1	4 (40.0)	4 (80.0)	2 (100%)	—				
AKI KDIGO 2	2 (20.0)	1 (20.0)	0 (0.0)	—				
AKI KDIGO 3	4 (40.0)	0 (0.0)	0 (0.0)	—				
Renal replacement therapy, No. (%)	3 (7.1)	1 (2.5)	0 (0.0)	OR	0.36 (0.02–3.38)	0.415	—	0.994
Invasive Ventilation, after randomization No. (%)	N = 41 8 (19.5)	N = 38 2 (5.3)	N = 41 3 (7.3)	OR	0.16 (0.018–0.962)	0.065	0.25 (0.11–1.25)	0.107
Invasive ventilation time, median (IQR)	11 (6–17)	16.0 (16–17)	5 (4–8)	MD	4.50 (−9.82 to 18.82)	0.553	-8.83 (−20.86 to 3.20)	0.184
Vasoactive agent, No. (%)	10 (23.8)	4 (10.0)	3 (7.1)	OR	0.38 (0.09–1.39)	0.161	0.29 (0.06–1.16)	0.098
Vasoactive agente time, days, median (IQR)	4 (2–7)	5 (2–8)	5 (4–9)	MD	-4.40 (−15.58 to 6.78)	0.455	-2.56 (−14.99 to 9.88)	0.694
ICU after randomization, No. (%)	N = 22 6 (27.3)	N = 27 0 (0.0)	N = 24 5 (20.8)	OR	—	0.993	0.69 (0.17–2.74)	0.601
Death	0 (0.0)	0 (0.0)	0 (0.0)	—				

MD = mean difference; OR = odds ratio.
Seven inpatients 28 days after admission | Four patients on oxygen dependence 28 days after admission.
[a] All models were adjusted for age.
[b] For invasive ventilation time and vasoactive agent time, the models are adjusted for age and randomization location.

3.2. Primary outcomes

Length of hospital stay at 28 days was significantly lower in both groups receiving propolis than in the standard care group, with a mean difference between the lower propolis dose and the control of −3.03 days (95% confidence interval [CI] −6.23 to −0.07; p = 0.049; median 7 versus 12 days), and for the higher dose of propolis compared to control it was −3.88 days (95% CI −7.00 to −1.09; p = 0.009; median 6 versus 12 days,) (Table 2). The cumulative frequency of discharge from the hospital is shown in Fig. 2a.

Patients assigned to propolis did not have a significantly different time on oxygen (with or without invasive ventilation) compared to the control group. The mean difference for the lower dose of propolis versus control was −2 days (95% CI −7.84 to 3.57; p = 0.470), and for the higher dose of propolis versus control it was - 0.99 days (95% CI −6.09 to 4.12; p = 0.710) (Table 2). The cumulative frequency of patients no longer on supplemental oxygen is shown in Fig. 2b.

3.3. Secondary outcomes

The incidence of acute kidney injury was 23.8%, 12.5%, and 4.8% for the control, 400 and 800 mg propolis/day groups, respectively. Only the higher dose group had a significantly lower rate of acute kidney injury than the control group (odds ratio [OR] 0.18; 95% CI 0.03–0.84; p = 0.048).

There were no significant differences in any of the remaining secondary outcomes. None of the patients taking the lower dose of propolis needed to be transferred to the intensive care unit, while the rate was 20.8% in the higher propolis dose group compared to 27.3% in the control (OR 0.69; 95% CI 0.17–2.74; p = 0.601) (Table 2). Thirteen patients initiated mechanical ventilation after randomization (5.3% of patients assigned the lower dose of propolis, 7.3% of those using the higher dose of propolis and 19.5% of the control group). The median number of days of invasive ventilation after randomization was 16 (16–17) in the group assigned to propolis 400 mg/day, 5 (4–8) in the propolis 800 mg/day group, and 11 (6–17) in the control group (Table 2).

The percentage of patients needing vasoactive agents was numerically lower in the groups receiving propolis than in the control group (10.0% with propolis 400 mg/day, 7.1% with propolis 800 mg/day, and 23.8% in the control group). The duration of vasoactive drug use and intensive care unit necessity was similar in all groups (Table 2). Laboratory data, including variation in serum levels of C-reactive protein over the seven days after randomization was recorded (Appendix Tables A1 and A2, Figs. A1 and A2).

3.4. Safety outcomes

Adherence to the trial intervention did not differ according to the treatment group. No patient had propolis treatment discontinued due to side effects. The percentages of patients experiencing adverse events did not differ significantly among the three groups. The most severe adverse event overall was shock/need for vasoactive drugs in 23.8% of the patients in the standard care group versus 10% in the propolis 400 mg/day group, and 7.1% in the propolis 800 mg/day group; p = 0.098. The second most common adverse event was acute respiratory failure, which occurred at a rate of 19.5% in the standard care group, 5.3% with propolis 400 mg/day, and 7.3% with propolis 800 mg/day (Table 2).

Gastrointestinal adverse events, specifically nausea, presented in one patient in the group with the lower dose of propolis and one patient in the control group. The only neurologic event was headache, and it presented in only one patient in the control group. The percentages of

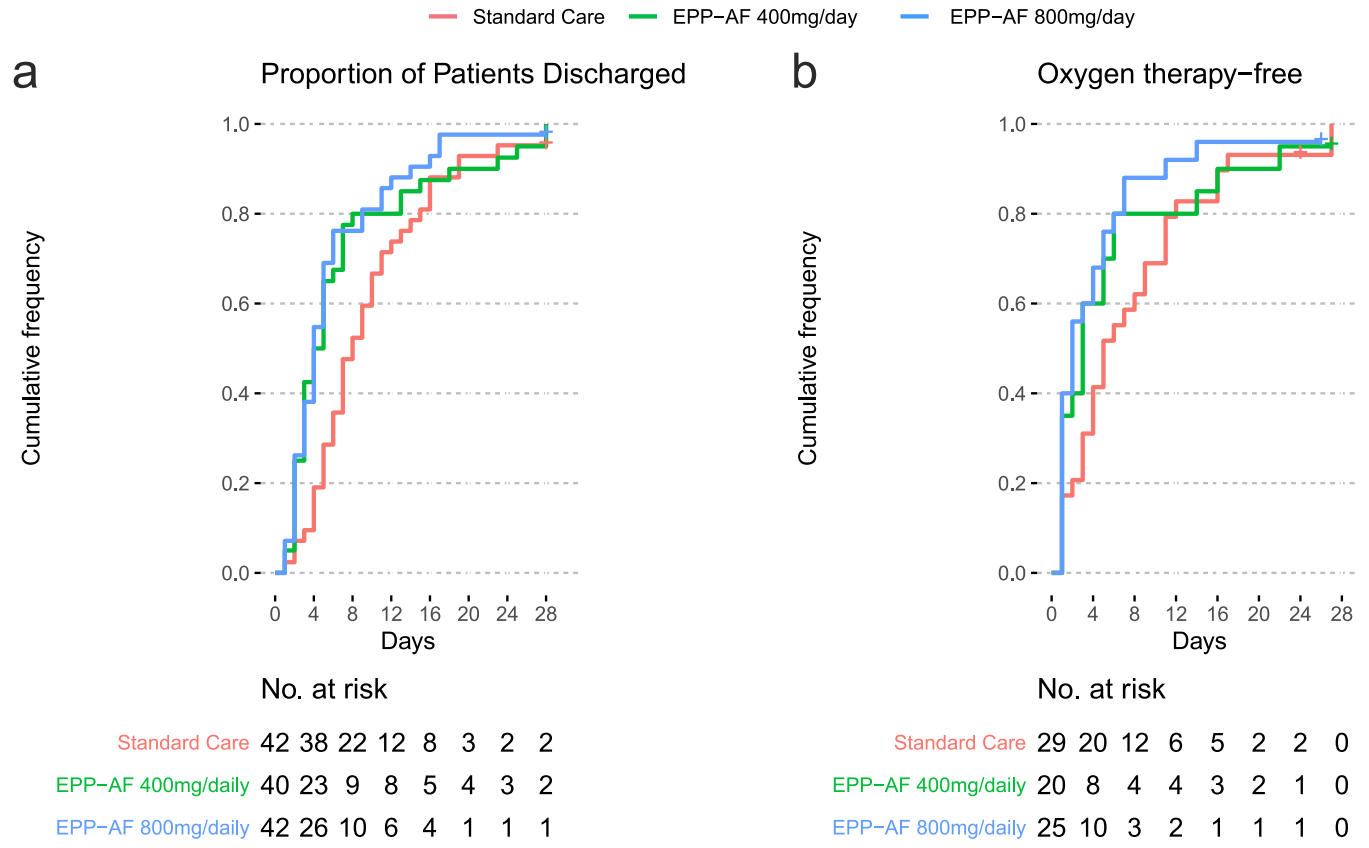

Fig. 2. Cumulative frequencies of COVID-19 patient primary outcomes. Time till discharge from the hospital (Fig. 2a) and time till oxygen-therapy free (Fig. 2b) for standard care and the two EPP-AF (propolis extract) doses.

patients with laboratory abnormalities were similar in the three groups (Appendix Table A1 and Appendix Fig. A1). Episodes of itching, an increase in alkaline phosphatase, and rash were not observed in any of the patients. No patient had propolis treatment discontinued due to any adverse event.

4. Discussion

Through this randomized clinical trial, we found that oral administration of propolis (EPP-AF®) for 7 days was safe and beneficial. Propolis plus standard care support was associated with a reduction in length of hospital stay after randomization for treatment, median 7 days (5–12) with 400 mg/day and 6 days (5–11) with 800 mg/day, compared with a median of 12 days (8–16) for standard treatment alone.

Severe pulmonary involvement is the most common problem associated with advanced cases of COVID-19 [51,52]. All 124 patients included in our study had some degree of pulmonary involvement, and just over half of them were on oxygen support at randomization, demonstrating that this patient population had moderate to severe cases of this disease. The time under oxygen support, including invasive and non-invasive therapy was not significantly different between the groups; the median in the 400 mg/day group was three days (1–6), and in the 800 mg/day group it was two days (1–5) ($p = 0.470$ and 0.710, respectively), compared with five days (3–11) for standard care alone. There was an apparent tendency for patients treated with propolis to have a reduced need for invasive oxygen therapy; but since relatively few patients in this cohort required this type of support overall, we cannot conclude that propolis was beneficial based on this clinical parameter.

We are not able to specify the mechanisms through which propolis acted in benefitting the COVID-19 patients. However, there is considerable evidence that various of the comorbidities associated with severe cases of COVID-19 can be ameliorated by propolis [9,18,41–45,53–56]. Also, quercetin, a propolis component, has demonstrated antiviral, cancer cell growth inhibition, thrombin inhibition and senolytic activities, which are all relevant properties for dealing with COVID-19 [9,57]. Caffeic acid, caffeic acid phenethyl ester (CAPE), apigenin and artepellin C are polyphenols found in propolis that can block the oncogenic kinase PAK1, which promotes an exaggerated and pathogenic immune response in advanced cases of COVID-19 [9]. Another propolis component, kaempferol, was found to inhibit the expression of TMPRSS2 and reduce ACE2 anchorage [13], which the virus requires to invade host cells.

Acute kidney injury is a common complication in COVID-19 [12,58]. It's incidence varies among COVID-19 patients, being associated with a poor prognosis, longer hospitalization times and greater mortality [51,58,59]. An observational study of more than 5000 hospitalized COVID-19 patients reported an overall frequency of 36% acute kidney injury; among patients on noninvasive oxygen support, the rate was 20%, and among those on mechanical ventilator support it was 89%. This implies a "cross talk" between lung and kidney under inflammatory insult [59,60].

In our study, patients treated with the higher dose of propolis had a significantly lower incidence of acute kidney injury compared to the control group. These findings have an important clinical significance, since acute kidney injury is associated with the worst outcomes, including greatly increased mortality [58,59,61]. The development of severe kidney lesions in COVID-19 patients is multifactorial, involving risk factors inherent to these patients (e.g., comorbidities), including volemic state, exposure to nephrotoxins, acute cardiac involvement (cardiorenal syndrome), systemic inflammation (immune response dysregulation; cytokine storm), endothelial lesions (microthrombi

formation), and renal tubular lesions [59,62]. EPP-AF promoted immunomodulation of the NF-kB/TLR4 system, with reduction of interleukins in renal tissue, helping protect the endothelium and mitochondria in a rat sepsis model [63]. The antioxidant, immunomodulatory and anti-inflammatory effects of propolis on the kidney, also apparent in another clinical trial [28], can help explain why the oral propolis adjunct treatment reduced kidney damage in the COVID-19 patients. Considering that many COVID-19 patients develop kidney damage and then require hemodialysis even after the disease course [61], this possibility of reducing the impact on the kidney would an important benefit of the use of propolis. Other relevant studies demonstrating the effectiveness of propolis for treating COVID-19 include a clinical trial of patients with uncomplicated upper respiratory tract infection [64], and a case study of a patient who had only mild symptoms after consuming Propomax® capsules, which contain EPP-AF® propolis [65].

Our study has several limitations. It was a single-center clinical trial, with only 40–42 patients in each treatment group, requiring greater caution in interpretations and generalizations concerning the findings. Although we blinded most of the health professionals involved in care of the patients, to reduce the possibility of interference, this trial was open. Also, the patients were followed for only a short period, limiting the possibility of evaluating long-term benefits.

In conclusion, the addition of oral propolis (EPP-AF®) to the standard care procedures was safe and had clinical benefits for the hospitalized COVID-19 patients, especially evidenced by a reduction in the hospitalization time. Possibly, administration early in the disease course would have an even greater benefit in reducing the disease's impact. Future studies can further assess the impact of propolis on renal protection in COVID-19 patients. Given our findings, and the evidence concerning the ways in which propolis can affect various disease mechanisms that are relevant to SARS-CoV-2 infection, propolis should be considered as an adjuvant in the treatment of COVID-19 patients.

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Author Contributions

MADS designed the trial and was the principal investigator, with overall responsibility for conducting the trial and for medical oversight of trial implementation and wrote the final report. RHP, SNFG, AVAM, JGR, and PBPB were responsible for the study design with the principal investigator. DDJ, AAB and RHP wrote the final report. JCR and TCS were responsible for analyzing the data. EBSG, RLASM, and TCA were responsible for coordinating and organizing the participants' data. SPS, LFMRC, MMDG, MBT, MHCAS, MOS, ML, SSU, and FSA contributed to the clinical and operational implementation of the trial. All authors contributed to trial design and interpretation of data and reviewed the final report.

Conflict of interest statement

Dr. Berretta is an employee of Apis Flora. Dr. Silveira and all the other authors, except for Dr. Berretta and Dr. De Jong, are employed by the São Rafael hospital, which is part of the D'Or Institute for Research and Education.

Data availability

Data supporting reported results that is not given here is available on request from the corresponding author. This data is not publicly available due to privacy requirements.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2021.111526.

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