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Original article

Effects of propolis and melatonin on oxidative stress, inflammation, and clinical status in patients with primary sepsis: Study protocol and review on previous studies

Naseh Pahlavani [a], Alireza Sedaghat [b], Ahmad Bagheri Moghaddam [c], Seyedeh Shabnam Mazloumi Kiapey [d], Jamshid Gholizadeh Navashenaq [e], Lida Jarahi [f], Reza Reazvani [d], Abdolreza Norouzy [g], Mohsen Nematy [d], Mohammad Safarian [d, **, 1], Majid Ghayour-Mobarhan [h, i, *, 1]

[a] Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Cardiac Anesthesia Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Department of Internal Medicine and Critical Care, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[e] Immunology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[f] Department of Community Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[g] Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
[h] Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[i] Cardiovascular Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

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SUMMARY

Background: Previous studies have explored the anti-inflammatory, anti-infection and oxidative stress reduction effects of propolis and melatonin in experimental studies. However, there are no studies at present exploring the effects of propolis and melatonin in patients with primary sepsis. The present study aims to evaluate the potential effects of propolis and melatonin as a pharmaceutical agent in patients with primary sepsis.

Methods/design: The study will be conducted as a randomized controlled clinical trial at the Imamreza hospital. Patients with primary sepsis, in four equal groups, will be recruited for the study. The treatment drugs are propolis and melatonin and the placebo. The following primary and secondary outcome measures will be evaluated: APACHE II Score, SOFA score, NUTRIC score, inflammatory factors, and oxidative stress markers.

Discussion: We describe the protocol for a clinical trial design evaluating the effects of simultaneous administration of propolis and melatonin in patients with primary sepsis. The result of the present study, positive or negative, should provide a step change in the evidence guiding current and future policies regarding the use of propolis and melatonin as an auxiliary treatment in patients with primary sepsis.

Trial registration: Iranian Registry of Clinical Trials: IRCT20181025041460N1. Registered on 6 November 2018.

1. Background

Sepsis is a potentially life-threatening condition caused by the host body's response to an infection that involves series of inflammatory, metabolic, hematological, and clinical responses which can lead to organ failure [1]. Due to the various definitions of sepsis, it is difficult to determine exact true prevalence of sepsis [2]. Primary sepsis, also named systemic inflammatory response syndrome (SIRS) were defined with four criteria include; tachycardia (heart rate >90 beats/

* Corresponding author. Metabolic Syndrome Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Vakil Abad Blvd., Opposite to Mellat Park, Mashhad 99199-91766, Iran. Fax: +98 5138002287.
** Corresponding author. Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Vakil Abad Blvd., Opposite to Mellat Park, Mashhad 99199-91766, Iran.
E-mail addresses: SafarianM@mums.ac.ir (M. Safarian), ghayourm@mums.ac.ir (M. Ghayour-Mobarhan).
[1] Mohammad Safarian and Majid Ghayour-Mobarhan contributed equally as corresponding author.

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min), tachypnea (respiratory rate >20 breaths/min), fever or hypothermia (temperature >38 or <36 °C), and leukocytosis, leukopenia, or bandemia (white blood cells >1200/mm^3, <4000/mm^3 or bandemia ≥10%) and patients who met two or more of these criteria fulfilled the definition of SIRS [3]. In one study, the prevalence of SIRS was 31%, with about half of it becoming sepsis [4]. Treatment for sepsis varies, depending on the site and cause of the initial infection, the organs affected and the extent of any damage, but generally included hemodynamic and metabolic resuscitation, antibiotic therapy, oxygen therapy, fluid therapy, and blood tests for patients monitoring [5].

Recently, interest has been increased in discovering new natural antimicrobial agents, and in most studies it has been shown that many natural compounds found in plants and spices have antimicrobial properties [6,7]. The inappropriate use of antibiotics has led to a dramatic increase in antibiotic resistance among communities and patients admitted to the hospital, hence, the use of natural compounds that have antibacterial properties is expanding and popularized and propolis is one of these natural ingredients [8]. Propolis is a natural resinous mixture similar to wax produced by honeybees from substances collected from parts of plants, buds, and exudates [9]. Nowadays, studies on propolis have increased due to its therapeutic and biological properties, and flavonoids in propolis have strong antioxidant properties that can remove free radicals and protect cell membranes from lipid peroxidation [10,11]. The propolis component varies according to its botanical origin; generally it contains more than 200 individual ingredients. Phenolic acids, esters, and flavonoids have been shown to account for most important of propolis composition and because of potential therapeutic use, greatly utilized by several cultures as a folk and alternative medicine [12,13]. Antimicrobial effects of propolis on gram-positive bacteria (such as staphylococci and streptococci) and gram-negative bacteria (*Escherichia coli*) have been shown in some studies [14]. Antibacterial activity of Propolis appears to be due to its flavonoids, such as Pinocembrin, Galangin and Pinobanksin [15]. So far, many studies have investigated the effects of propolis on inflammatory, glycemic and oxidative factors in various diseases [16,17], but its effects have not yet been studied in patients with SIRS criteria admitted to the intensive care units.

Melatonin (N-acetyl-5-methoxy-tryptamine) is synthesized from the tryptophan amino acid in the pineal gland and is secreted into the cerebrospinal fluid [18]. In the human bodies, melatonin participates in various functions including the regulation of sleep, reproduction, mood, promotion of antioxidant defense, immunomodulation, and as an anti-inflammatory agent [19]. It has been shown that melatonin has antioxidant, anti-tumor, and anti-inflammatory effects [20,21]. In one study 20 mg melatonin for 3 days, lead to improve the rate and symptoms of sepsis in newborns [22]. But so far no study has been done on the effects of melatonin on the symptoms and severity of primary sepsis (SIRS) in adults admitted to the intensive care units.

Hence, this study has been designed to investigate the efficacy of propolis and high dose of melatonin administration in the patients with systemic inflammatory response syndrome (SIRS) that hospitalized in intensive care unit via a four parallel-group randomized double-blind placebo-controlled clinical trial and mini-review on previous studies.

2. Methods/design

This protocol was written according to the CONSORT SPIRIT 2013 guidelines [23]. The flowchart of trial enrollment, interventions and assessments is presented in Fig. 1. Study visits will be scheduled for days 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. A framework of the time schedule of enrollment, interventions, assessments, visits, and an overview of the timeline of the study interventions and assessments is provided in Table 1.

2.1. The goals and hypotheses

2.1.1. General aim

The general aim of the present study is to determine the effects of propolis supplementation and high doses of melatonin effects on oxidative stress indices, inflammatory factors and clinical status through a double-blind, randomized, controlled clinical trial in patients with Systemic Inflammatory Response Syndrome (SIRS) in Intensive Care Unit.

2.1.2. The specific aims and hypothesis

Hypothesis: we hypothesize that the infection in the patients with primary sepsis who are treated with propolis and high dose of melatonin in addition to conventional treatment (antibiotic therapy) its will be better than that of the control group treated only with antibiotics. We also hypothesize that other sepsis-related parameters APACHE II Score (Acute Physiology and Chronic Health Evaluation II), SOFA score (Sequential Organ Failure Assessment), NUTRIC score (NUTrition RIsk in Critically ill), inflammatory factors, malondialdehyde (MDA), total antioxidant capacity (TAC), C-reactive protein (CRP), interleukin 6, interleukin 1β, and interleukin 10 will be improved in the treatment groups in comparison to the control group.

Aim 1

To determine the effects of propolis and high dose melatonin supplementations on APACHE II score, and SOFA score in patients with primary sepsis.

Aim 2

To determine the effects of propolis and high dose of melatonin supplementations on inflammatory factors, oxidative stress indices, and total antioxidant capacity levels in patients with primary sepsis.

Aim 3

To determine the effects of propolis and high dose of melatonin on anthropometric parameters, and NUTRIC score in patients with primary sepsis.

2.2. Study design and setting

The study will be a phase-I/II, randomized, double blind clinical trial. It will be conducted at the Imam Reza Hospital at Mashhad University of Medical Sciences of Iran for a period of 6 months from February 2019, assessing the effects of the daily supplementation of propolis and high dose of melatonin containing in patients with systemic inflammatory response syndrome (primary sepsis). We will include 44 pre-sepsis as determined by the sepsis campaign criteria (fever or hypothermia (temperature >38 or <36 °C), tachycardia (heart rate >90 beats/min), tachypnea (respiratory rate >20 breaths/min), and leukocytosis, leukopenia, or bandemia (white blood cells >1200/mm^3, <4000/mm^3 or bandemia ≥10%). Patients who met two or more of these criteria fulfilled the definition of SIRS, and primary sepsis was defined as infection or suspected infection leading to the onset of SIRS [24]. The patients will be selected from the individuals with primary sepsis hospitalized in intensive care units of Imam Reza Hospital.

2.3. Sample size

To estimate the sample size, based on a previous study [25], we used interleukin 6 (IL-6) as a key variable and considered type one (α) and type two errors (β) of 0.01 and 0.10 (power = 90%), respectively. We reached 9 patients in each group. Assuming 20%

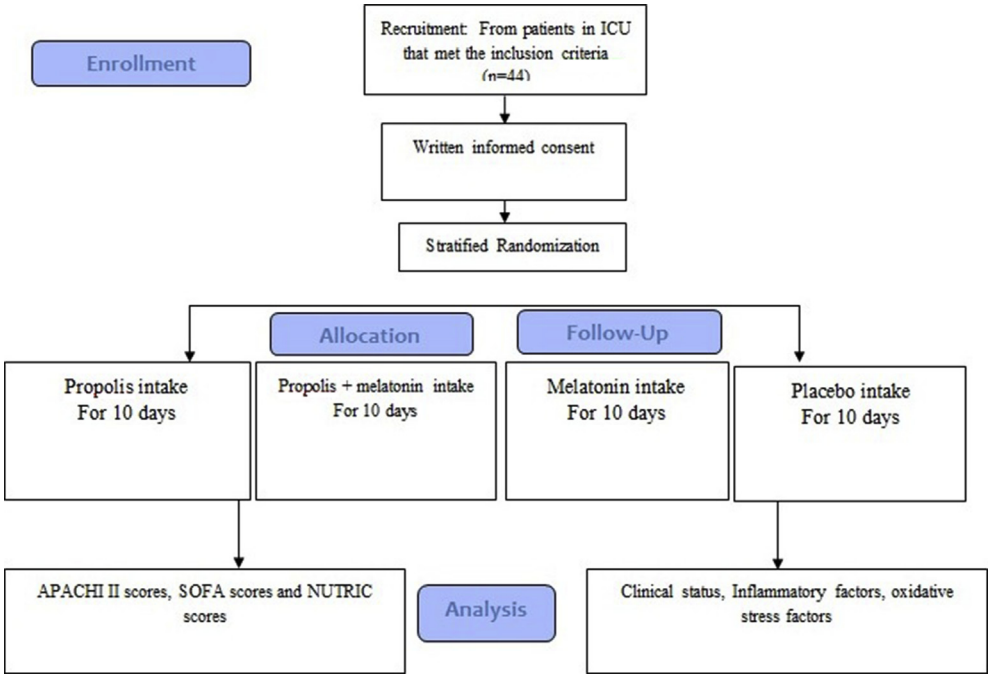

Fig. 1. Study design schematic.

dropouts in each group, the final sample size was determined to be 11 patients per group by the formula given below;

$$n = \frac{\left(Z_1 - \frac{\alpha}{2} + Z_1 - \beta\right)^2 \left(\sigma_1^2 + \sigma_2^2\right)}{\left(\mu_1 - \mu_2\right)^2} \quad (1)$$

2.4. Randomization and blinding

Randomization for the 4 parallel treatment arms will be carried out by the principal investigator after checking the inclusion and exclusion criteria. The individuals will be stratified at randomization based on, age (below 45 years and 45 years and over), gender and the severity of the disease (APACHE II scores 0–24 and 25 and more) to ensure equal distribution of these variables in the four

Table 1
Schedule of enrollment, intervention, and assessment of the clinical trial.

Timeline	Study period				
	Enrollment	Allocation	Post-allocation		Follow-up
		Day 1	Day 5	Day 10	28 days after intervention
Enrollment:					
Eligibility screen	×				
Informed consent	×				
Demographic date	×				
Blood culture	×				
Allocation		×			
Interventions					
Group 1		⟷	⟷	⟷	
Propolis + Melatonin placebo					
Group 2		⟷	⟷	⟷	
Propolis + Melatonin					
Group 3		⟷	⟷	⟷	
Melatonin + Propolis placebo					
Group 4		⟷	⟷	⟷	
Propolis placebo + Melatonin placebo					
Primary assessment:		×			
APACHI II score, SOFA score, NUTRIC score, Inflammatory indices, Oxidative stress indices					
Middle assessment:			×		
APACHI II score, SOFA score					
Last assessment:				×	
APACHI II score, SOFA score, NUTRIC score, Inflammatory indices, Oxidative stress indices					
Follow up					×

arms. The randomization sequence would be generated using the SPSS statistical software package (version 18.0). The investigators and patients are blind to the treatment allocations. The medication will be delivered in similar packets and labels, each with its own sequence number. The allocation sequence number will be generated by one of the investigators not involved in managing patients.

2.5. Statistical analysis

To examine the normal distribution of variables, we used Kolmogorov–Smirnov test. The analyses were conducted based on intention-to-treat (ITT) approach. One-way analysis of variance (ANOVA) (or multi-variable covariance analysis (ANCOVA)) was used to detect differences in general characteristics, APACHE II score, SOFA score, NUTRIC score, dietary intakes and biomarkers of inflammation and oxidative stress at study baseline between the four groups. If the distribution of data is abnormal, for the between groups comparison Kruskal–Wallis test, and for the inter-groups comparison the Wilcoxon test will be used. The *P* values less than 0.05 will be considered statistically significant.

2.6. Inclusion and exclusion criteria

Eligibility criteria for the participants are presented in Table 2. Nurses, nutritionists and research team will perform the intervention. The participants will be randomized into four equal groups using a blocking stratified sampling method by age and sex and APACHE II score using a computerized table of random digits.

2.7. Study groups

2.7.1. Treatment groups

To evaluate a dose–response relationship, the phase-II study will include three treatment groups. The groups will receive propolis doses of either 1000 mg or melatonin 20 mg or propolis (1000 mg/day) and melatonin (20 mg/day) based on results from previous research and the placebo group will receive the same placebo (Fig. 2.)

1. Group 1 – propolis with dose 1000 mg daily
2. Group 2 – propolis (1000 mg/day) plus melatonin (20 mg/day)
3. Group 3 – melatonin with dose 20 mg daily Control group
4. Group 4 – placebo

2.8. Outcomes

2.8.1. Primary outcomes

Primary outcomes assessed would be the clinical status, inflammatory indices, APACII II score, SOFA score and NUTRIC.

2.8.2. Secondary outcomes

Oxidative stress index and anthropometric indices will be considered as secondary outcomes.

2.9. Safety assessment index

The following information will be recorded/measured for the safety assessment: vital signs (daily), blood culture (at the beginning of the study), inflammatory markers (at the beginning and end of the study), oxidative stress indices (at the beginning and end of the study), APACHE II questionnaire (initially, middle and end of study), SOFA questionnaire (initially, middle and end of study), general medical examination (at the beginning of the study), and NUTRIC score questionnaire (at the beginning and end of the study).

2.10. Procedure

2.10.1. Recruitment

Patients will be recruited on a voluntary basis (consent to participate by themselves or by their first degree relatives) from a hospital who is attending the critical care wards with having inclusion criteria in Imam Reza hospital in Mashhad University of Medical Sciences.

2.11. Measurement tools

2.11.1. Anthropometric parameters

Measuring the mid-arm circumference (MAC) to assess malnutrition and the weight will be measured with the Balas bed scale, length of forearm (ulna) to will be used to calculate the patient

Table 2
Eligibility criteria for the effects of propolis and high dose of melatonin supplementation on SIRS patients.

Inclusion criteria
Patients: men and women 18–75 years old
Admission to the intensive care unit with the criteria of systemic inflammatory response syndrome (primary sepsis)
Having two or more criteria of SIRS and proven infection by blood cultures
Fill out the informed consent form by the patient or first-degree relatives of the patient
Glasgow Coma scale $\geq$ 7
Non-entry criteria
Pregnancy and lactation
Patients who are not allowed to start nutrition support in the first 24–48 h.
Autoimmune disorders
Cancers
Severe sepsis
Chemotherapy and radiotherapy in the past month
Intake positive inotropic drugs include dopamine, dobutamine and epinephrine
Severe and active bleeding
Morbid obesity (BMI > 40)
Infection with Human Immunodeficiency Virus (HIV)
Severe liver failure
Patients with an food allergy
Exclusion criteria
Create any non-entry criteria
Unwillingness to continue the study
Sensitivity to supplements (propolis and melatonin)

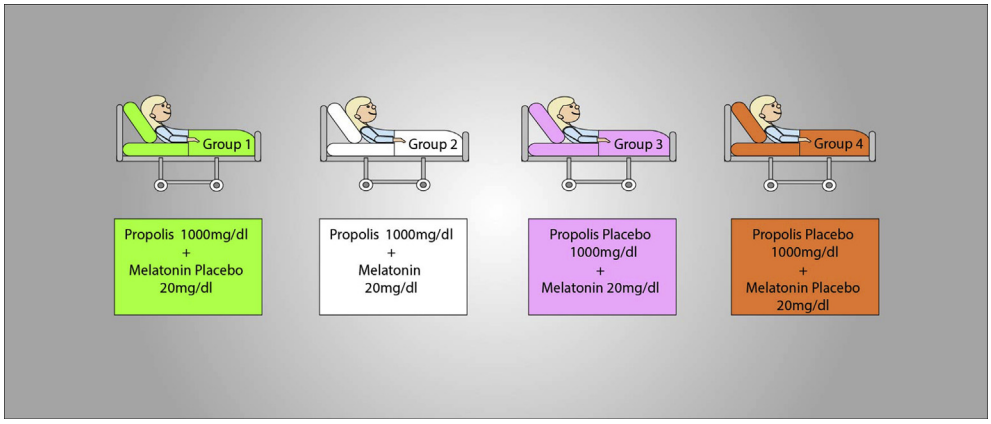

Fig. 2. Four study groups; treatments and placebo.

height [26]. All anthropometric measurements will be made by trained nutritionist.

2.11.2. Dietary measurements

The nutritional status of all patients will be evaluated by using the NUTRIC score questionnaire at baseline. The caloric goal will be calculated by using the Harris Benedict Equation × 1.3, taking into consideration the information inserted (such as age, weight and height). To feed the patients by enteral nutrition, a hospital formulation formula with a specific caloric intake will be used, and nutrition confounder will also be adjusted. The amount of energy requirement of each patient will be calculated based on need, nutritional factors and stress coefficient, and the required formulation will be provided through enteral nutrition.

2.11.3. Biochemical assay

Blood samples will be obtained for routine laboratory testing on a daily basis. Severity scores, including the Sequential Organ Failure Assessment score (SOFA), and Acute Physiology and Chronic Health Evaluation II (APACHE II) score, will be measured with questionnaire and automatically calculated by the web-based system, taking into consideration the individual values inserted for each patient to avoid possible human errors in the calculations of the severity scores. Serum interleukin-6 (IL-6), interleukin 1β, and interleukin 10 will be measured by commercial ELISA kits from BD Biosciences at the beginning and end of the study. Serum malondialdehyde (MDA) equivalents will be assayed spectrophotometrically by the reaction with thiobarbituric acid [27]. Pro-oxidant–antioxidant will be measured based on protocol [28].

3. Discussion

Up to now, the effects of propolis, and melatonin have been discussed separately on infection and inflammation, but there is little information available that if given both simultaneously, what will affect the process of infection and inflammation in hospitalized patients. In addition, the potential benefits of these biopharmaceuticals in pre-sepsis subjects who are at high risk for developing SIRS to advanced sepsis and septic shock have received little attention. This trial will address this important research gap, by exploring and comparing the role of propolis and melatonin in modifying inflammation, infection, and clinical status among pre-sepsis (SIRS with proven infection) patients.

3.1. Antibacterial effects of propolis

Propolis has been used in folk medicine as a treatment for some infectious diseases, and in recent studies its antimicrobial effects have been studied and its anti-bacterial, antifungal, anti-fungal, and antiviral effects have been shown [29,30]. One study showed that the combination of propolis from different locations could reduce the rate of infection caused by Staphylococcus, *Escherichia coli* and Candida albicans [31]. Previous studies have shown that propolis can improve the infection of diabetic foot ulcers, normalize levels of interleukins 1β and 6, and TNF-α [32,33]. Another study showed that Propolis had synergistic effects on anti-TB drugs such as streptomycin, isoniazid and rifampicin [34]. Propolis antimicrobial effects are probably due to the ability of propolis in cell lysis and cell membrane damage, as well as inhibition of the ability to move, protein synthesis and cell division in bacteria with effect on RNA polymerase and these effects probably due to the presence of phenolic compounds in Propolis is such as caffeic acid, terpenes, flavonoids and esters [35–37]. Therefore, it seems that the administration of propolis with the addition of melatonin in patients with systemic inflammatory response syndrome (primary sepsis) can help reduce infection, inflammation and also improve the treatment process.

3.2. Effects of melatonin on inflammation and oxidative stress

In previous studies, most of which have been performed on animal samples, the effects of melatonin on inflammation and oxidative stress factors have been shown to some extent [38–40]. In one animal study, the use of melatonin in mice with septic shock with dose of 10 mg/kg twice daily for 3 days can reducing pro-inflammatory factors of TNF-α and IL-6 [41]. In Alamili et al. study, 100 mg melatonin (infusion) for 8 h, was able to reduce the level of IL-1β as a pro-inflammatory factor, but its effects on levels of TNFα, IL-6 and malondialdehyde (oxidative stress marker) were not significant [38]. In Gitto et al. study, taking melatonin at a dose of 20 mg/day for 72 days in neonates with septic shock could reduce the levels of C-reactive protein, white blood cells and lipid peroxidation, and ultimately reduce infant mortality [42]. In another study, supplementation of 10 mg/kg body weight of melatonin for 7 days in newborns with respiratory distress syndrome decreased lipid peroxidation, reduced nitrite and nitrate levels, and decreased the TNF-α and interleukin 6 and 8 [43]. In one study, administration of melatonin at a dose of 10 mg/day for 30 days reduced levels of malondialdehyde and increased glutathione peroxidase levels in obese patients [44]. In another study, the

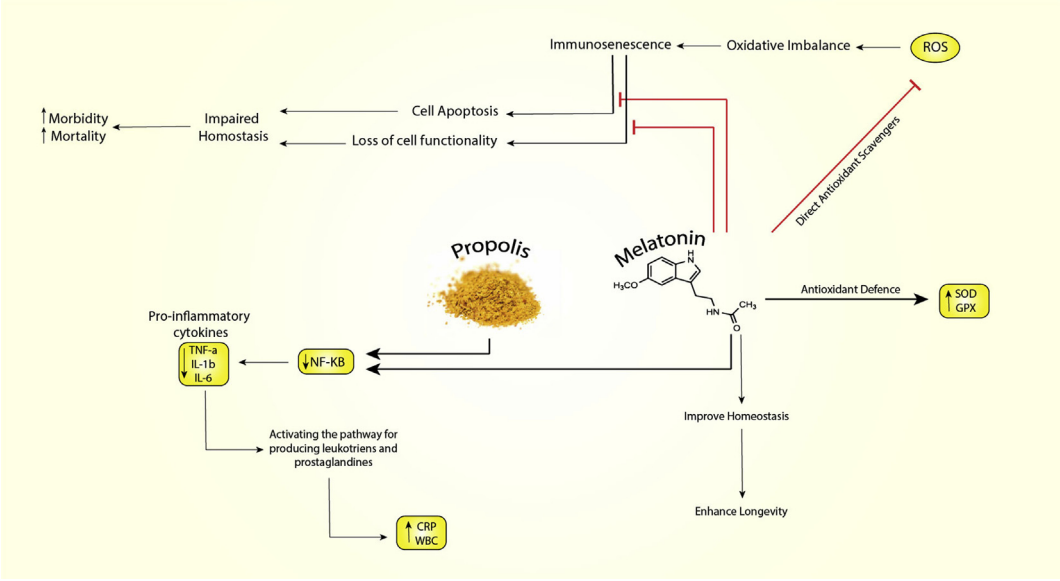

Fig. 3. Probably mechanism of propolis and melatonin effects in inflammation, and oxidative stress. Figure Abbreviation: ROS, Reactive Oxygen Species; NF-κB, Nuclear Factor-Kappa B; SOD, Super Oxide Dismutase; GPX, Glutathione Peroxidase; TNF-α, Tumor Necrosis Factor Alpha; IL-1β, Interleukin 1 beta; IL-6, Interleukin 6; CRP, C-Reactive Protein; WBC, White Blood Cell.

administration of melatonin at a dose of 3 mg/day for 3 months in patients with chronic obstructive pulmonary disease reduced oxidative stress (8-isopropranes) [45]. In previous studies, different mechanisms have been proposed for the effects of propolis and melatonin on the process of infection, inflammation and oxidative stress [46–48]. According to previous studies, co-administration of propolis and melatonin appears to reduce the rate of infection, inflammation and oxidative stress levels (Fig. 3). Therefore, it can be expected that giving these two supplements (propolis and melatonin) simultaneously can lead to faster recovery, shorten the length of stay in hospital, and reduce mortality in patients admitted to the intensive care unit.

4. Conclusion

Considering that in previous studies the effects of propolis and melatonin have been shown separately on the improvement of inflammatory, oxidative stress and infection rates, therefore, the aim of our study will be to evaluate the effects of simultaneous administration of propolis and melatonin on infection rate, oxidative stress, Inflammatory markers, clinical status, and mortality rates in patients with systemic inflammatory response syndrome.

Ethics approval and consent to participate

The study protocol has been assessed and approved by the research ethics committee of Mashhad University of Medical Sciences (approval number: IR.MUMS.MEDICAL.REC.1397.290) and conformed to the Declaration of Helsinki. The study has been registered at irct.ir as IRCT20181025041460N1. Important protocol modifications will be sent to relevant parties by the investigators.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request. The supporting data are available.

Authors' contributions

NP, MS, AS, AB and MGM conceptualized the study. NP, MGM and MS are the main researchers. LJ developed the statistical design. All of the authors have seen and approved the final version of the protocol.

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Conflict of interest

The authors declare no other competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2019.06.007.

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