The Effects of Melatonin and Propolis on Markers of Inflammation, Oxidative Stress, Clinical Outcomes, and Survival Rate in Patients with Primary Sepsis Hospitalized in Intensive Care Unit: A Randomized Clinical Trial

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Research

Keywords: Systemic inflammatory response syndrome, Sepsis, Propolis, Melatonin, Inflammation, Oxidative stress, Clinical outcome

DOI: https://doi.org/10.21203/rs.3.rs-56718/v1

Abstract

Background: Systemic Inflammatory Response Syndrome (SIRS) that occurs under stressful conditions affecting all organs of the body. Previous studies have shown that propolis and melatonin have the potential to improve inflammation and oxidative stress, so the aim of this study was to investigate the effects of these supplements on SIRS treatment.

Method: This was a randomized, controlled clinical trial in SIRS patients comprising 55 subjects that were randomly assigned to 3 intervention or control groups. In the 3 intervention groups, patients were treated with propolis alone (at dose of 1000 mg/day), propolis (1000 mg/day) plus melatonin (20 mg/day), and melatonin alone (20 mg/day) respectively, while there was no intervention in the control group. The inflammatory and oxidative stress markers and clinical outcomes were measured before and after of the intervention, also 28-day survival rate was assessed.

Results: Propolis plus melatonin reduced serum interleukin 6 ($p = 0.001$) and CRP levels ($p < 0.001$), and was associated with an increased gavage intake ($p = 0.016$). At the end of the study, there was no difference between the groups in the oxidative stress and hematological indices. In the propolis+melatonin group, the clinical outcomes were significantly improved ($p < 0.05$). Also the SOFA score between the groups did not differ at any time, its changes was significant during the time ($p > 0.001$). The average survival after 28 days of follow-up in the propolis, propolis+melatonin, melatonin and control groups were 24.08, 25.69, 22.05 and 19.42 days respectively, although this was not statistically significant ($p=0.07$).

Conclusion and relevance: Supplementation with propolis+melatonin may help to improve clinical outcomes by reducing inflammation and was probably associated with an increase in the calorie intake, leading to an increase in the survival rate in SIRS patients, although more studies are necessary to prove these effects.

Trial registration: IRCT20181025041460N1.

Introduction

Systemic inflammatory response syndrome (SIRS) is an inflammatory response in all organs of the body that occurs under stressful conditions for the body and may be of infectious origin, or of non-infectious origin (burns, trauma, and other factors). If the cause of this syndrome is infection it is classified as sepsis (1). If the SIRS of infectious origin (primary sepsis) is not treated and controlled, it can lead to advanced sepsis, septic shock, and ultimately Multiple Organ Dysfunction Syndrome (MODS) (2). The World Health Organization (WHO) has identified sepsis as a major health problem that its incidence has continued to increase despite declining mortality (3). SIRS and sepsis are common causes for the hospitalisation of patients especially in the intensive care unit (ICU), and its prevalence varies depending on the underlying disease and the level of nursing and medical care (4), for example, its prevalence in one study in Japan was 84%, and one study showed that more than 36,000 deaths occurred per year from sepsis in the UK (2). The prevalence of severe sepsis in the ICU was reported to be 12.4 percent (5). In Iran, there has been no complete study of the prevalence of SIRS, but in one study, the mortality rate from SIRS with an infectious origin of 4.5% and the mortality rate due to sepsis were reported to be 9.9% in ICU (6). Due to the epidemiology of SIRS an early diagnosis, timely, and appropriate interventions in this syndrome can prevent it from progressing to advanced sepsis, septic shock, and MODS and be essential key to reducing mortality with increases the likelihood of survival of these patients (2), however despite the advances in the understanding of the pathophysiological basis of sepsis, clinical diagnosis is still limited to medical history, symptomatic examination, and non-specific laboratory and hemodynamic criteria (7). Some factors such as aging, multidrug resistance, and immunosuppressive agents can increase the incidence of sepsis and due to the fact that the treatment of these patients is often with antibiotics and anti-inflammatory drugs, in most cases, drug resistance occurs and the treatment of sepsis slows down and can move to more advanced stages (3, 5, 8). A diverse set of molecules and oxidative stress are involved in the expression and side effects of a SIRS, so neutralizing these pre-inflammatory factors (such as interleukin 1, tumor necrosis factor alpha, reactive oxygen species (ROS), reactive nitrogen species (RNS), and interleukin 6) can lead to reduce the burden of disease and mortality (9, 10). Various studies have shown that oxidative stress in SIRS patients is shown by reduced plasma values of total radical-trapping antioxidant parameter (TRAP) and its components (uric acid, sulfur group (SH) proteins, non-conjugated bilirubin, vitamin C, vitamin E and other plasma antioxidants) and also high levels of malondialdehyde (MDA), therefore, reducing oxidative stress by adjuvant therapies along with the main and common treatments can be very helpful in treating these patients (8, 11).

Recently, there has been increasing interest in discovering new natural antimicrobial agents, and most studies have shown that many natural compounds found in plants and spices have antimicrobial properties (12, 13). Propolis is a natural product that is a wax-like substance made from products derived from bees, that is composed of various substances that have been studied today due to its therapeutic and biological properties (14, 15). The flavonoids in propolis have strong antioxidant properties that are able to remove free radicals and protect cell membranes from lipid peroxidation and seems the antibacterial activity of propolis appears to be due to the presence of flavonoids such as Pinocembrin, Galangin, and Pinobanksin (16, 17). Another important component of propolis, and that has antioxidant properties, is caffeic acid phenyl ester (CAPE), which inhibits the production of oxygen free radicals (18). Various studies have shown that propolis can reduce the factors associated with oxidative stress and inflammation (19, 20). On the other hand, many studies have shown that melatonin has cellular protective effects such as; it regulates oxidative stress, apoptosis and mitochondrial homeostasis, as well as regulates the immune system (21). In addition, animal studies have shown the protective effects of melatonin as a supplement in bacterial sepsis and shock, and these effects have been linked to some melatonin functions such as; its antioxidant, cellular, and immune-boosting effects (22, 23). Urinary excretion of some melatonin metabolites, such as 6-sulfate melatonin (aMT6) have been seen in the ICU patients, as well as nightly melatonin plasma levels was inversely related with the severity of the disease in patients with severe sepsis in these patients (24, 25).

Therefore, according to the pathogenesis of systemic inflammatory response syndrome and the antibacterial and anti-inflammatory effects of propolis, as well as the antioxidant and anti-inflammatory function of melatonin, we decided to evaluate the effect of these two substances in SIRS patients hospitalized in the ICU, so that it may be presented to these patients as a new adjuvant treatment.

Methods

Study design

The trial was a single center, prospective, randomized, controlled trial that conducted in critically ill patient with primary sepsis between December 2018 and October 2019 in the Intensive Care Units, Imamreza Hospital. The study protocol and consent forms were approved by the Research Ethics Committee of Mashhad University of Medical Sciences (registration code: IR.MUMS.MEDICAL.REC.1397.290) and was registered in the Iranian Registry of Clinical Trials (registration code: IRCT20181025041460N1) (26). Written informed consent was obtained from all patients or their legal representatives before inclusion in the study.

Patients and interventions

Fifty five patients were randomly assigned to four groups of the study using block randomization to propolis (n=14), propolis plus melatonin (n=14), melatonin (n=14) or control groups (n=13). Patients who had the eligibility criteria include age 18 to 75 years, admission to the intensive care unit with the criteria for systemic inflammatory response syndrome (primary sepsis), providing written, informed consent form by the patient or first-degree relatives (legal representatives) of the patient and the Glasgow coma scale (GCS) is equal to 7 or higher entered to this study. Exclusion criteria were; pregnancy and lactation, patients who are not able to start nutritional support in the first 24-48 hours, autoimmune disorders, cancer, severe and advanced sepsis, chemotherapy and radiotherapy in one last month, received positive inotropic drugs (including dopamine, dobutamine, and epinephrine), severe liver failure, severe and active bleeding, human immunodeficiency virus (HIV), known food allergies, and morbid obesity (body mass index (BMI)> 40). In addition all selected patients were treated based on the sepsis guideline (27), and at any time of the study, participants were excluded from the study if they were reluctant to continue the study, any of the criteria for non-entry, or sensitivity to melatonin or propolis supplements.

The intervention groups in addition to the usual treatments, received 1000 mg/day propolis alone, 1000 mg/day propolis plus 20 mg/day melatonin, or 20 mg/day melatonin alone, respectively for the 10-day, however, in the control group, only routine treatments were provided.

The combination of aqueous and ethanolic extract propolis was purchased as syrup from Soren Tech Toos Company (Soren tech-Toos Co, Mashhad-Iran, Batch Number; STT5.006) and melatonin was purchased from Amin Pharmaceutical Company (Aminpharma Co, Isfahan, Iran). The melatonin-receiving groups received 20 mg of melatonin daily (in two divided doses of 10 mg at noon and 3 hours before bedtime) and groups receiving propolis daily received 1000 mg of propolis (in two divided doses in the morning and evening) for 10 days.

Data collection and measurements

A checklist was designed by the research team in which information including age, gender, underlying disease, medical history; drug history, etc. were collected and recorded. In the present study inflammatory markers and clinical outcomes assessed as primary outcomes and also oxidative stress markers, infection indices, gavage intakes, and 28-day survival assessed as secondary outcomes.

Clinical and nutritional outcomes measurements

Anthropometric measurements including estimated height based on ulna length and patient age (28), weight (using the patient's bedside scale (Seca-Germany)), body mass index (BMI) were calculated using the estimated height and weight of the patients (Kg/m^2 where Kg is a patient's weight in kilograms and m^2 is their height in meters squared), and also mid arm circumference (MAC) using non-elastic tape meters for each patient was measured at the beginning of the study.

Nutritional support of patients using enteral nutrition with hospital base formula that has a certain amount of calorie, macro and micronutrients was used (testing by TESTA Quality Control Laboratory, Mashhad-Iran), Enteral nutrition through the nasopharyngeal tube was started using a hospital gavage at a rate of 25 ml/hour and increased at the same rate every 6 hours according to the patient's tolerance to achieve the desired calories and the volume of gastric residue was not significant (300 ml>). The calorie requirement of each patient was calculated by hospital nutrition experts and the research team based on 25 kcal/kg actual body weight and the required protein content was considered to be 1.5 g/kg ideal body weight.

The NUTrition Risk in the Critically ill (NUTRIC score) Questionnaire was used at the beginning and end of the study to assess nutritional status and estimate malnutrition of the patients (29).

To estimate the risk of mortality and severity classification of the disease, the Acute Physiology and Chronic Health Evaluation II (APACHE II) questionnaire was used at the beginning and after the intervention (30), and Sequential Organ Failure Assessment (SOFA) questionnaire was also used to evaluate the function of the organs of the body during the study on days 1, 5, and 10 of the study (31). Patients were also followed up from the beginning of the study to 28 days after the intervention to assess mortality rate.

Hematological and Inflammatory markers measurements

The white blood cell count was measured by Sysmex K-1000 Hematology Analyzer-Japan and then the differential counts were performed under a microscope to accurately measure neutrophil values before and after the intervention. The serum CRP level was measured using the immunomodibrometry method and the quantitative CRP detection kit (Bionik, Iran) with the BT3500 (Biotecnica Instruments SpA -Italy) auto-analyzer and also, the measurement of interleukin 6 by ELISA method using kit (Demeditec-Germany) was performed in the beginning and end of the study.

Oxidative stress markers measurements

In the beginning and end of the study to assess the pro-oxidant-antioxidant balance (PAB), Hamidi Alamdari et al.'s method was used (32) and also malondialdehyde levels using kit (Zell Bio-Germany) was measured.

Sample size and statistical analysis

Sample size calculation was made based on 80% power and an alpha error of 5% to detect the inflammation effect based on changes in IL-6 by propolis supplementation using the method of Zhao and colleagues study using the below formula (20).

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

Based on the above formula, the sample size was at least 9 patients in each group and due to the high probability of dropout in the intensive care unit, at least 13 patients entered in each group.

All analyses were performed on an intention-to-treat (ITT) basis using the statistical package for social sciences (SPSS, Inc., Chicago, IL, USA) software version18. The normal distribution of variables was evaluated using the Shapiro-Wilk test and based on the results of this test, all data were normally distributed. In this study, chi-square test was used to compare the qualitative variables, we used paired samples t-test to assess the effects of propolis and melatonin supplements on primary and secondary outcomes measurements within groups, and the ANOVA test to compare between groups changes and, if necessary, post hoc tests followed by the Tukey comparison test was used. Also we adjusted the variables based on their baseline values by using Analysis of Covariance (ANCOVA). $p < 0.05$ is considered statistically significant.

Results

A total of 106 patients were considered for inclusion in the study, of whom 55 subjects were selected based on inclusion and exclusion criteria. From 55 patients at baseline, two patients in the propolis group (due to hospital discharge and death), two patients in the propolis+ melatonin group (due to hospital discharge and personal reason), one patient in the melatonin group (due to death), and one patient in the control group (due to death) not completed the study. Therefore, 49 patients (propolis (n = 12), propolis+ melatonin (n=12), melatonin (n=13), and control (n = 12)) completed the trial (Figure 1). Mean age, weight, estimated BMI, estimated height, and MAC of patients were 58.87 ± 17.83 years, 62.81±10.12 kg, 21.83±3.23 kg/m^2, 169.78±10.61 cm, 28.01±4.05 cm respectively. There was also no statistically significant difference between the baseline demographic and clinical characteristics of the participants in the four study groups (Table 1). There were no statistically significant differences between the baseline supplement and medications intakes in the four groups of the study (Table 2). The baseline dietary intakes based on hospital gavage (energy, macronutrients and some micronutrients) of patients are presented in Table 3. No significant differences were found in usual intake of energy, carbohydrate, protein, fat, zinc, iron, selenium, vitamin C, vitamin E, calcium, and copper between the four groups.

At the beginning of the study, there were no significant differences between hematological factors, oxidative stress and inflammatory indices between the 4 study groups. After 10-day intervention the levels of WBC and neutrophils in the propolis and propolis+ melatonin groups significantly decreased compared to the baseline, however, these changes were not significant compared with the four groups (Table 4).

After 10-day intervention, serum IL-6 (p=0.01) and CRP (p=0.002) levels between the 4 group of the study were significantly decreased. The serum IL-6 and CRP levels were significantly lower in the propolis and propolis+ melatonin groups compared with baseline, also the changes in serum IL-6 in the propolis+ melatonin group was significant compared with other 3-groups, and CRP changes in propolis+ melatonin group was significant compared to melatonin and control groups (Table 4).

Although serum MDA levels in the propolis + melatonin group decreased significantly after intervention compared to baseline, but this change were not significant compared to other 3-groups. PAB changes before and after the intervention was not significant in either group (Table 4).

At the beginning of the study, there were no significant differences between clinical indices including APACHE II score, NUTRIC score, and gavage intakes between the 4 study groups. The APACHE II and NUTRIC score in the propolis, propolis+ melatonin, and melatonin groups were significantly decreased after 10-day intervention, but the APACHE II score changes in the comparison between the groups remained significant only in the propolis + melatonin group compared to the other 3 groups and NUTRIC score changes only in the propolis + melatonin group compared to the control group was significant (Table 5). The rate of gavage intake only in the propolis+ melatonin group significantly increased compared with baseline (p=0.001) and this change compared to melatonin and control groups was remain significant, the amount of gavage intake in other 3-groups of study were not significant after the intervention (Table 5).

The SOFA score before the interventions, at day 5 and, after the interventions were measured. Although SOFA score levels decreased at the end of the study in the three groups of propolis, propolis + melatonin, and melatonin compared to before the intervention, but in none of these stages did SOFA changes significantly, although over time (Time × group), that changes has been significant (p>0.001) Table 6 and Figure 2.

Adjusted means of dependent variables changes according to baseline values among four groups of the study were shown in table 7. In addition, analyses with adjustment of baseline values, WBC, neutrophils, PAB, and MDA throughout the study revealed no significant changes in our observed findings (Table 7), also IL-6, CRP, APACHE II score, SOFA score, and gavage intake changes after adjustment remain significant (Table 7).

After 28 days of follow-up, the average survival rate (Kaplan-Meier curve) in the propolis group was 83.3%, propolis + Melatonin group 76.9 %, melatonin group 46.2 %, and 41.7% in control group (Figure 3).

The mean survival in patients in 28 days of follow-up in the control group was 19.42 days (14.88-23.95) with a confidence interval (CI) of 95%, propolis group 24.8 days (19.13-29.04 with 95% CI), propolis+ melatonin group was 25.69 days (22.58-28.80 with 95% CI), and was 22.05 days (18.11-26.01with 95% CI) in the melatonin group. Although the survival rate was higher in the propolis and propolis + melatonin groups compared to other groups, there was no statistical significant difference between the 4 groups of the study using log rank test (p=0.07).

Discussion

We have found that administration of 1000 mg propolis with 20 mg of melatonin daily for 10 days improved inflammatory factors and clinical status in patients with primary sepsis in the intensive care unit, while, these effects on oxidative stress markers, WBC, neutrophils and 28-day mortality were not statistically significant.

Gitto et al., have reported that a daily oral dose of 20 mg of melatonin in neonatal infants with sepsis reduced serum CRP levels (33), although in our study co-administration of melatonin and propolis reduced CRP levels, but melatonin alone did not have a significant effect on CRP levels, possibly due to the dose received at 20 mg/kg body weight in infants is higher than in adults and this may have been more effective. Melatonin at a dose of 10 mg/kg for 7 days was reported to reduce the production of pro-inflammatory cytokines such as interleukin 6, interleukin 8, and TNF-α in infants with respiratory distress syndrome (34). Some other clinical trials have shown the significant effects of melatonin on reducing inflammatory factors (35-37), while a number of other studies have not shown significant effects on reducing inflammation (38). These contradictory effects can be due to differences in the study population, the type of participant's disease and the dose and duration of supplementation. It has been suggested that melatonin may reduce the expression of sticky molecules such as ICAM and inhibit the transfer of NF-kB into the nucleus, thereby reducing the inflammatory process and lowering the level of inflammatory factors (39, 40). In the present study, propolis supplementation alone and in combination with melatonin was able to reduce the levels of inflammatory factors. In line with our study, propolis (1000 mg daily) for 12 weeks in diabetic patients was able to significantly reduce CRP and TNF-α levels (41). Other clinical trials have shown that propolis at doses of 830 mg to 1,500 mg in some chronic disease can significantly reduce the levels of inflammatory factors (20, 42, 43), while lower doses of propolis did not effect on the inflammation levels (44). Therefore, with increasing propolis dose, the amount of its active ingredients also increases, which leads to inhibition of the activity of the NF-κB signaling pathway and ultimately causes anti-inflammatory effects of propolis (45, 46).

Animal studies have shown that melatonin can reduce lipid peroxidation, increase glutathione levels, and reduce neutrophil infiltration into tissues (47, 48). Gitto et al., showed that melatonin could decrease lipid peroxidation and malondialdehyde levels (33). In another study, melatonin intakes was able to reduce lipid peroxidation and nitrite and nitrate levels in infants with respiratory distress syndrome (34). The results of these studies are inconsistent with our results, which was probably due to these studies have been performed on infants and receiving similar amounts of melatonin can have a greater impact on them. In another study, melatonin supplementation at a dose of 10 mg/day for 12 weeks in diabetic patients significantly increased plasma glutathione and decreased malondialdehyde levels (35), and the inconsistency of the results of this study with our results is probably due to the longer duration of the intervention than our study, as well as the type of disease of the participants. Melatonin also inhibits mitochondrial damage caused by sepsis by restoring disrupted antioxidant systems, increasing glutathione levels and the activity of the glutathione reductase enzyme, inhibiting nitrite formation and inducing the expression of mitochondrial nitric oxide enzymes, in addition to melatonin can decreased lipid peroxidation in mitochondria of various tissues (49).

Hesami et al. have reported that daily supplementation with 1500 mg of propolis for 8 weeks could reduce ox-LDL levels in patients with type 2 diabetes, that the results of this study are contrary to the our findings, which is probably due to higher doses of propolis as well as long duration of their study (50). Also in a similar study, Afshapour et al. showed that a 1500 mg dose of propolis for 12 weeks significantly increased the activity of superoxide dismutase and glutathione peroxidase, as well as increasing the total antioxidant capacity in the diabetic patients (42). However, inline with our results in two studies 900 mg/day propolis did not affect malondialdehyde levels and the activity of the superoxide dismutase enzyme, but increased the activity of the glutathione peroxidase enzyme, which low effects may be due to different types and locations of propolis extraction (20, 51).

We found that propolis alone, and in combination with melatonin, was able to significantly reduce the levels of WBC and neutrophils after interventions compared to baseline, however, these changes were not significant between the four groups. In one study, 20 mg of melatonin for 3 days was able to decrease infection in infants with sepsis and also melatonin intake at a dose of 20 mg/day for 72 hours in infants with septic shock reduced the rate of infection and WBC (33, 52). Melatonin blocks the NF-κB signaling pathway from lipopolysaccharides by inhibiting nuclear displacement and DNA binding activity from one of the NF-κB subunits (53). In general, it can be concluded that a decrease in the secretory rhythm of melatonin as a result of bacterial infections may be effective in sepsis severity, while giving a melatonin supplement can clearly reduce the severity of this infection (53). A review study have reported the antibacterial activity of propolis and its extracts against gram-positive and gram-negative bacterial strains and found that propolis has antibacterial activity against a wide range of gram-positive bacteria, but this activity is more limited in the gram-negative bacilli (54). An animal study showed that propolis could reduce the rate of infection caused by Salmonella bacteria, as well as keep WBC and neutrophil in normal levels (55). In one animal study that conducted by El-Aidy et al., propolis was able to maintain leukocyte and neutrophil at normal levels compared to other groups (56). Although in one animal study, propolis extract did not have a significant effect on the infection caused by Mycobacterium tuberculosis in guinea pigs (57). Therefore, the antibacterial effects of propolis probably depend on variables such as the dose of propolis used, the location of the collected propolis, its content of polyphenolic compounds and the solvents used to extract it, and antibacterial effects of propolis are mainly due to the its effect on the division of bacterial cells, disruption of the cell wall and cytoplasmic membrane of bacteria (54, 58).

In our study supplementation with propolis and melatonin improves clinical outcomes and survival rate, so far, no studies have measured the effects of propolis and melatonin supplements directly on these scores, but some components of these questionnaires have been studied in previous reports. In the

study that carried out by Thanoon et al. on healthy individuals, propolis supplementation at dose of 1 g per day for 2 months reduced blood pressure as well as decreased blood uric acid levels (59). the probably reason for the effect of lowering blood pressure was due to the propolis effects on the levels of the tyrosine hydroxylase enzyme (limiting enzyme in the biosynthesis of catecholamines), as well as an increase in the production of vascular nitric oxide (60). Also in confirmation of our findings another studies have shown positive effects of propolis on the improving organ functions (44, 61, 62). Animal studies have shown positive effects of melatonin on survival, mortality, and duration of mechanical ventilation (53, 63, 64). Melatonin supplementation reduced the pain and mechanical ventilation time in premature infants compared with the control group (65). The protective effects of melatonin are probably related to inhibition of the apoptotic process caused by sepsis, reduction of oxidative damage, and possibly reduction of the inflammatory response (22).

A study in hemodialysis patients showed that appetite decreased with increasing levels of inflammatory indicators such as CRP and IL-6 (66). In one animal study, intra- peritoneum injection of 4 doses of melatonin increased food intake in the rats (67). Regarding the effects of propolis on food intake, the results of the previous studies are contradictory (20, 59, 68). So far, no studies have examined the co-administration effects of these two supplements on dietary or gavage intake, because the increase in inflammation is related to the reduction in food intake it can be concluded that due to the fact that the rate of inflammation in the propolis+ melatonin group has decreased significantly compared to other groups, this increase is more likely in the gavage intakes due to the reduction of inflammation (69, 70).

The strengths of the present study include: the design of the classified blocks randomization was used to randomly assign participants to 4 groups of the study that this scheme led to the homogeneous distribution of features between the groups and the control of the confounders, and second strengths in this study, for the first time, the co-administration effect of propolis and melatonin supplements in an acute condition and in critically ill patients was measured. The limitations of the present study were the inability to measure all the factors predicted in the study protocol (26), and small sample size due to financial constraints.

Conclusions

The present study showed that daily consumption of propolis at a dose of 1000 mg plus 20 mg melatonin for 10 days in pre-sepsis patients was able to reduce inflammation and subsequently improve organ function and decrease the dysfunction of vital organs, as well as associated with the increase of gastrointestinal tolerance and finally, it was accompanied by an increase in the amount of gavage intake. In our study, despite the increased survival rate, its effects were not statistically significant. It is recommended that multicenter studies with a higher dose, longer duration and large sample size in critically ill patients with the same underlying disease be conducted.

Declarations

Acknowledgments

This paper was adapted from PhD. Thesis, which was supported by Faculty of Medicine, Mashhad University of Medical Sciences (code: 970487). So, we thank the authorities of this university for their cooperation. Also, we appreciate the patients and their family who participated in the study.

Author contributions

NP, AS, GAF, ABM, MS, and MGM designed the research. NP, AS, MM, MH, ABM, SSMK, AN, RR, MS, and MGM performed the trial, NP, JGN, GAF, MFN, MM, SF, LJ, HT, and MGM analyzed and interpreted the data and drafted the manuscript. All of the authors read and approved the final manuscript.

Funding

There was no funding for the research reported.

Availability of data and materials

All data was added as an SPSS file and can be made available to applicants

Ethics approval and consent to participate

This project was approved by the ethics committee of Mashhad University of Medical Sciences and was registered with code IR.MUMS.MEDICAL.REC.1397.290, and informed consent obtained from all patients or 1st degree relatives to participate in the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Baseline demographic and clinical characteristics of the four study groups

Variables	Propolis [a] (n=14)	Propolis+ Melatonin [b] (n=14)	Melatonin [c] (n=14)	Control [d] (n=13)	P value
Age (y)	58.21±15.76	57.92±20.43	60.92±17.73	58.38±19.02	0.97
Sex (Female %)	50	57.1	42.9	53.8	0.88
Weight (Kg)	64.35±8.63	60.98±11.46	62.59±12.83	63.36±7.25	0.85
MAC (cm)	29.81±3.83	26.89±3.82	26.88±4.75	28.51±3.29	0.16
Estimated BMI (Kg/m^2)	22.52±2.68	21.11±3.64	21.03±3.34	22.76±3.14	0.35
GCS	9.21±2.04	8.22±1.18	9.28±1.86	8.31±2.01	0.25
Underlying Disease (%)					
COPD or other respiratory problems	71.4	64.3	78.6	69.2	
Cardiovascular Problems	0	0	0	7.7	
Stroke	7.1	0	0	0	
Infection Disease	7.1	7.1	7.1	0	0.75
Gastrointestinal Problems	14.3	28.6	14.3	23.1	
Other Diseases	0	0	0	0	
Educational attainment (%)					
Illiterate	35.7	28.6	21.4	15.4	
Elementary	28.6	50	42.9	38.5	
Diploma	28.6	14.3	35.7	23.1	0.71
Bachelor	7.1	7.1	0	15.4	
Masters and Doctorate	0	0	0	7.7	
Marital Status (%)					
Single	21.4	14.3	7.1	7.1	0.64
Married	78.6	85.7	92.9	92.3	

Abbreviation; MAC, Mid Arm Circumference. GCS, Glasgow Coma Scale. COPD, Chronic Obstructive Pulmonary Disease. MS.c, Master of Science.

Quantitative variables reported as Mean±SD using One-way ANOVA

[a] Received daily syrup containing 1000 mg Propolis daily

[b] Received daily syrup containing 1000 mg Propolis plus 20 mg Melatonin powder daily.

[c] Received 20 mg Melatonin powder daily

[d] Control group (without intervention)

Table 2. Patient's supplement and medications intakes in the four groups of study in the baseline

Variables	Propolis [a] (n=14)	Propolis+ Melatonin [b] (n=14)	Melatonin [c] (n=14)	Control [d] (n=13)	P value
Vitamin C (%)	7.1	7.1	7.1	7.1	0.84
Vitamin E (%)	0	7.1	0	7.7	0.54
Vitamin D (%)	7.1	14.3	21.4	7.7	0.64
Vitamin B-complex (%)	0	0	7.1	7.7	0.54
Zinc sulfate (%)	14.3	7.1	21.4	38.5	0.21
Multivitamin (%)	21.4	7.1	14.3	7.7	0.64
Omega 3 (%)	0	7.1	7.1	7.7	0.78
Albumin (%)	0	7.1	0	7.7	0.99
Apotel (%)	7.1	7.1	7.1	7.7	0.99
Prednisolone (%)	0	0	0	7.7	0.34
Dexamethasone (%)	7.1	0	0	7.7	0.54

All data obtained from chi-squared test

[a] Received daily syrup containing 1000 mg Propolis daily

[b] Received daily syrup containing 1000 mg Propolis plus 20 mg Melatonin powder daily.

[c] Received 20 mg Melatonin powder daily

[d] Control group (without intervention)

Table 3. Energy, macro and micronutrients intake in the four groups of study in the baseline

Variables	Propolis [a] (n=14)	Propolis+ Melatonin [b] (n=14)	Melatonin [c] (n=14)	Control [d] (n=13)	P value
Energy (Kcal)	957.14±391.67	1057.14±377.16	925.01±286.72	1046.15±229.54	0.65
Carbohydrate (g)	140.77±54.96	148.01±52.81	145.01±56.23	164.76±53.89	0.68
Protein (g)	33.02±13.02	35.94±12.82	35.21±13.65	40.01±13.08	0.58
Fat (g)	34.92±13.53	37.01±13.21	36.25±14.05	41.18±13.47	0.66
Zinc (mg)	6.67±2.72	6.86±2.45	6.81±2.61	7.64±2.51	0.74
Iron (mg)	5.14±1.98	5.49±1.96	5.38±2.08	6.12±2.01	0.63
Selenium (µg)	31.57±12.23	33.83±12.06	33.15±12.84	37.67±12.31	0.62
Vitamin C (mg)	82.05±30.52	89.14±29.37	84.91±32.94	96.49±31.58	0.65
Vitamin E (mg)	8.34±3.24	8.98±3.21	8.81±3.41	10.01±3.21	0.61
Calcium (mg)	702.14±275.13	761.14±271.56	724.28±240.58	847.38±277.15	0.51
Copper (µg)	535.53±207.23	570.85±203.66	559.28±216.88	635.53±207.86	0.64

All data obtained from One-way ANOVA test

[a] Received daily syrup containing 1000 mg Propolis daily

[b] Received daily syrup containing 1000 mg Propolis plus 20 mg Melatonin powder daily.

[c] Received 20 mg Melatonin powder daily

[d] Control group (without intervention)

Table 4. Hematological indices, inflammatory factors, and oxidative stress marker measurements at study baseline and 10 days after the intervention in the four groups of the study

Variables	Propolis group [a]			Propolis + Melatonin group [b]			Melatonin group [c]			Control group [d]	
	Baseline (n=14)	After (n=12)	p-Value*	Baseline (n=14)	After (n=12)	p-Value*	Baseline (n=14)	After (n=13)	p-Value*	Baseline (n=13)	After (n=12)
WBC (10*3/µL)	13.3±4.7	10.1±4.8	0.007	15.4±5.6	9.6±3.1	0.001	11.5±4.7	9.7±3.5	0.22	14.7±3.5	13.3±4.
Neutrophils (%)	85.5±6.5	78.7±9.5	0.02	85.8±9	80.1±9.7	0.01	81.1±9.6	80.2±8.2	0.57	81.7±7.8	80.1±9.
Serum IL-6 (pg/ml)	309.1±44.3	289.1±64.8	0.06	309.9±45.1	Ω, Φ, η 256.7±47.6	0.001	338.6±37.9	328.9±44.4	0.21	315.3±61.6	311.5±5
Serum CRP (mg/L)	40.6±18.1	29.3±15.6	0.001>	46.7±17.4	η, Ω 25.1±14.1	0.001>	52.6±17.4	49.7±23.2	0.55	44.8±13.9	44.2±15
Serum PAB (HK)	180.7±53.3	152.1±76.1	0.31	173.5±57.8	162.9±71.7	0.81	154.4±71.6	137.3±81.5	0.51	170.3±60.8	162.4±7
Serum MDA (nmol/L)	15.1±6.7	12.1±3.7	0.053	15.3±5.3	11.2±4.8	0.006	15.4±6.3	12.2±2.6	0.51	12.2±5.2	12.9±4.

Abbreviations: WBC; White Blood Cells. IL-6; Interleukin 6. CRP; C-reactive Protein. PAB; Pro-oxidant-Antioxidant Balance. MDA; Malondialdehyde

[a] Received daily syrup containing 1000 mg Propolis daily

[b] Received daily syrup containing 1000 mg Propolis plus 20 mg Melatonin powder daily.

[c] Received 20 mg Melatonin powder daily

[d] Control group (without intervention)

All values are means± SDs

*Obtained from Paired t-test

**Obtained from One-way ANOVA (†= Baseline, #=After Intervention)

Tukey's test:

[Ω] Significant compared to the control group

[Φ] Significant compared to the propolis group

[η] Significant compared to the melatonin group

Table 5. APACHE II score, NUTRIC score, and gavage intakes measurements at study baseline and 10 days after the intervention in the four groups of the study

Variables	Propolis group [a]			Propolis + Melatonin group [b]			Melatonin group [c]			Control grou
	Baseline (n=14)	After (n=12)	p-Value*	Baseline (n=14)	After (n=12)	p-Value*	Baseline (n=14)	After (n=13)	p-Value*	Baseline (n=13)
APACHE II score	23.3±5.8	19.1±5.5	0.03	23.8±7.1	Φ, η, Ω 15.5±7.3	0.001>	23.9±5.3	20.6±4.1	0.001>	21.9±8.1
NUTRIC score	5.3±1.5	Ω 4.1±1.4	0.01	5.2±2.5	Ω 2.9±2.1	0.001>	5.3±1.9	4.8±1.4	0.002	4.5±2.1
Gavage intake (ml)	957.1±391.6	1050.1±368.6	0.32	1057.1±377.1	η, Ω 1479.1±392.2	0.001	925.1±286.7	1026.9±179.6	0.42	1046.1±229

Abbreviations: APACHE II; Acute Physiology and Chronic Health Evaluation II. NUTRIC score; NUTrition Risk in the Critically ill.

[a] Received daily syrup containing 1000 mg Propolis daily

[b] Received daily syrup containing 1000 mg Propolis plus 20 mg Melatonin powder daily.

[c] Received 20 mg Melatonin powder daily

[d] Control group (without intervention)

All values are means± SDs

*Obtained from Paired t-test

**Obtained from One-way ANOVA (†= Baseline, #=After Intervention)

Tukey's test:

Ω Significant compared to the control group

Φ Significant compared to the propolis group

η Significant compared to the melatonin group

Table 6. SOFA score measurements at study baseline, day 5, and 10 days after the intervention in the four groups of the study

	Propolis [a]	Propolis+ Melatonin group [b]	Melatonin group [c]	Control group [d]	p-Value*
Before Intervention	9.8±2.9	9.7±2.2	9.2±2.9	9.1±1.6	0.85
Day 5	9.3±2.3	8.7±2.3	9.4±1.7	9±0.9	0.81
After Intervention	7.6±2.1	7.1±1.7	8.1±1.5	10.1±2.3	0.054
Time × group**					0.001>**

Abbreviations: SOFA; Sequential Organ Failure Assessment.

[a] Received daily syrup containing 1000 mg Propolis daily

[b] Received daily syrup containing 1000 mg Propolis plus 20 mg Melatonin powder daily.

[c] Received 20 mg Melatonin powder daily

[d] Control group (without intervention)

All values are means± SDs

*Obtained from One-way ANOVA

**Obtained from Repeated measure test

Table 7. Adjusted means of dependent variables changes according to baseline values among four groups of the study

Groups Variables	Propolis group [a] (n=12)	Propolis+ Melatonin group [b] (n=12)	Melatonin group [c] (n=13)	Control group [d] (n=12)	p-Value*
Serum IL-6	-24.43±9.38	-59.56±9.37 [Φ, η, Ω]	-4.71±9.11	-4.19±9.37	0.001>
Serum hsCRP	-15.41±3.91	-21.25±3.88 [η, Ω]	-0.79±3.78	-1.08±3.88	0.001
WBC	-3.02±1.04	-4.08±1.04	-2.15±1.01	-0.89±1.05	0.18
Neutrophils	-5.67±2.29	-4.11±2.29	-2.12±2.21	-2.26±2.28	0.66
Serum PAB	-20.08±22.48	-8.28±22.36	-31.62±21.71	-8.36±22.34	0.85
MDA	-2.42±2.15	-2.99±2.14	-2.24±2.06	-0.46±2.18	0.86
APACHE II	-3.69±1.03	-8.21±1.03 [Φ, η, Ω]	-3.86±1.01	-0.82±1.04	0.001>
NUTRIC score	-1.12±0.29 [Ω]	-2.13±0.29 [η, Ω]	-0.69±0.28	0.16±0.29	0.001>
SOFA	-2.26±0.53	-2.49±0.53 [Ω]	-1.67±0.51	0.56±0.53	0.001
Gavage intake	67.58±69.76	404.88±70.23 [Φ, η, Ω]	42.68±67.01	72.96±69.53	0.002

Abbreviations: WBC; White Blood Cells. IL-6; Interleukin 6. CRP; C-reactive Protein. PAB; Pro-oxidant-Antioxidant Balance. MDA; Malondialdehyde. APACHE II; Acute Physiology and Chronic Health Evaluation II. NUTRIC score; NUTrition Risk in the Critically ill.

[a] Received daily syrup containing 1000 mg Propolis daily

[b] Received daily syrup containing 1000 mg Propolis plus 20 mg Melatonin powder daily.

[c] Received 20 mg Melatonin powder daily

[d] Control group (without intervention)

All values are means± standard error

The means for the baseline values of the dependent variables have been adjusted.

*Obtained from One-way ANOVA

Changes: After the intervention values -Before the intervention values

Tukey's test:

[Ω] Significant compared to the control group

[Φ] Significant compared to the propolis group

[η] Significant compared to the melatonin group

Figures

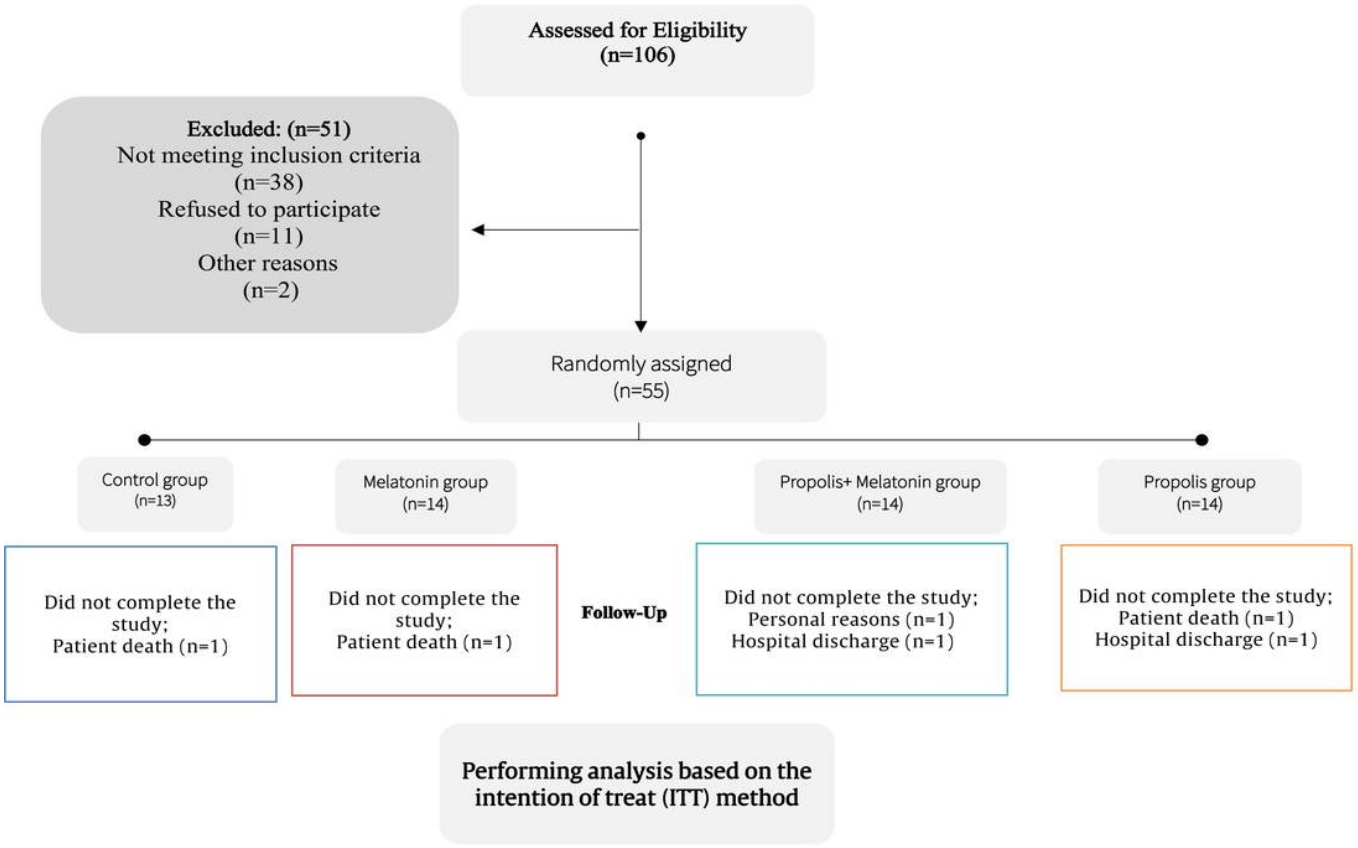

Figure 1

Summary of patients flow.

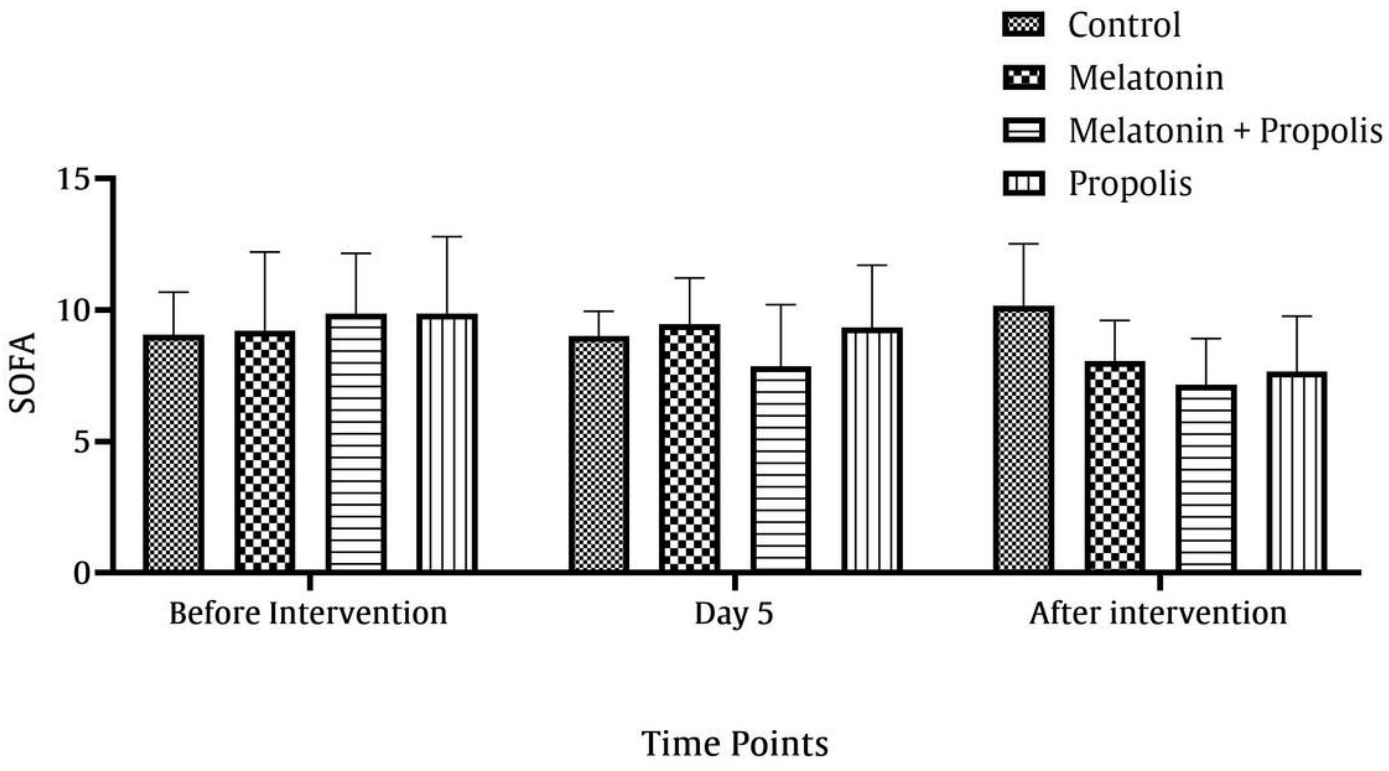

Figure 2

SOFA score changes during the trial (Before, Day 5, and After the intervention) in four groups of the study.

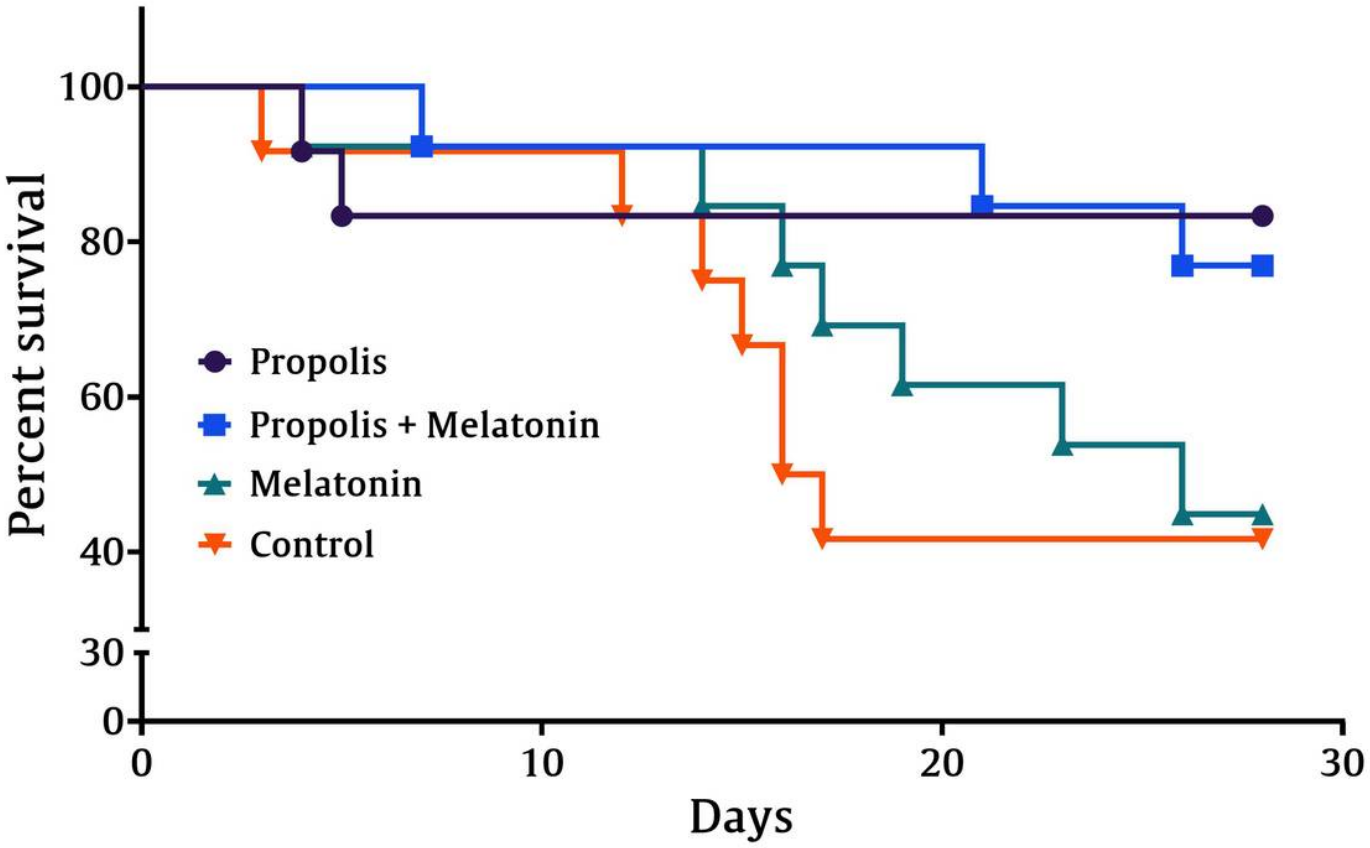

Figure 3

Kaplan-Meier curve of 28 days survival, comparing the four groups of the study. No significant difference was found.