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Original Research

N-Chromosome Royal Jelly, Propolis and Bee Pollen Supplementation Improve the Clinical Conditions of COVID-19 Patients: A Randomized Controlled Trial

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Abstract

Royal jelly, propolis, and bee pollen are used for different purposes all around the world according to their anti-inflammatory, antioxidant, and antimicrobial activities. Given that Coronavirus 2019 (COVID-19) is a viral condition accompanied by a dysregulated inflammatory response in the body, we intend to evaluate the effects of natural supplementations on the disease course. A randomized, open-label, controlled trial was conducted among 50 definitive cases of COVID-19. These patients were randomly assigned into control and intervention groups. Royal Jelly, propolis, and bee pollen were prescribed to patients in the intervention group (n = 24) in addition to conventional treatment; while the control group only received the standard treatment (n = 26). At the end of the study, functional class improved in both groups, but this change was more pronounced in the intervention group ($p < 0.05$).

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Moreover, total symptoms duration and the time to return to work were significantly reduced in the intervention group ($p < 0.05$). Although royal jelly, propolis, and bee pollen are not definitive treatments in COVID-19 patients, they can be used as an adjuvant treatment to limit disease symptoms and virus propagation.

Keywords: Royal jelly; Bee pollen; Propolis; Hyperinflammation; Corona virus disease 2019 (COVID-19)

Introduction

Coronavirus disease 2019 (COVID-19) is a new type of highly contagious viral infection that led to a global pandemic and has become a significant threat to the existence of present human society. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes hyperinflammatory tissue-damaging and immunothrombosis with the induction of cytokine storm. These events are thought to be major causes of end-organ damage, including respiratory failure and death [1,2]. So, the development of vaccines and antiviral agents is urgent. In this regard, a variety of therapeutic options including antiviral drugs (e.g., remdesivir), anti-SARS-CoV-2 monoclonal antibodies (e.g., bamlanivimab/etesevimab, casirivimab/imdevimab), anti-inflammatory drugs (e.g., dexamethasone), and immunomodulator agents (e.g., baricitinib, tocilizumab) are available under Food and Drug Administration (FDA) issued Emergency Use Authorization (EUA) or being evaluated in the management of COVID-19 [3]. Also, some vaccines including BNT162b2, mRNA-1273, Ad26.COV2.S, and ChAdOx1 nCoV-19 vaccine, have been developed indigenously and have been approved or granted emergency use authorization to prevent COVID-19 in many countries around the world [4]. None of the mentioned drugs has yet been confirmed for the treatment of COVID-19 patients [5]. In addition to conventional medicine, complementary medicine makes use of natural products, and numerous studies have focused on their effect against infectious diseases [5]. The development of synthetic techniques led to a significant reduction in the importance of natural products that are important for the development of new drugs [6]. One of the most important natural products is honey, which has been used for different medicinal purposes since ancient times, and scientists also accept it as a new effective medicine for many kinds of diseases [7]. Honey products, have some effective components such as flavonoids, phenolic acids, phenolic compounds, terpenes, and have been used in ancient traditional medicines to treat various conditions called apitherapy. These products have offered an alternative treatment for a variety of acute and chronic diseases such as cancer, diabetes, infectious, cardiovascular, neurological, gastrointestinal disorders [8], and also exhibit antimicrobial, antioxidant, immunomodulatory, and anti-inflammatory activities [9].

Several studies have shown that a hyperinflammatory immune response or cytokine release syndrome (CRS) is observed in critically unwell patients with SARS-CoV-2 [2,10]. So, in

this article, we intend to evaluate the immunomodulatory effects of honey bee products in addition to antiviral treatment on COVID-19 patients as a primary objective and laboratory findings as secondary objectives.

Materials and Methods

Study design

This randomized, open-label, controlled trial, included participants with definite COVID-19 confirmed by clinical examination, radiological and laboratory studies, enrolled for screening between April and September 2020.

All procedures performed in the study involving human participants were under the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of the National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences (IR.SBMU.NRITLD.REC.1399.048). Registered IRCT number is IRCT20200209046427N1.

Participants

The inclusion criteria for this study were as follows: (1) definite COVID-19 diagnosis, (2) age between 18 and 90 years, (3) without significant psychological disorder, (4) time to admission less than 48 h, and (5) proper patient compliance. Exclusion criteria were as follows: (1) patients who took warfarin, (2) patients with a history of allergy or reaction to bee products. All of the patients signed informed consent according to the medical ethics committee.

Randomization and masking

At the beginning of the study, the enrolled patients were randomly allocated into 2 groups: an intervention group consists of 24 cases and the control group consists of 26 patients. The intervention group was given three products including N-chromosome royal jelly (Caspian Apiaries Company), propolis (Caspian Apiaries Company), and mixed honey (Caspian Apiaries Company). They used at least one teaspoon of each of them, twice a day with water or milk or fruit juice in addition to their standard treatment [11]; while the control group received their standard treatment only. The random assignment was a blocked design so that ABAB arrangement was selected at random and the four participants were assigned accordingly. The study was unblinded to the treating clinicians and the patients.

Study protocol

A data collection form was completed for each patient separately. In all subjects, demographic data such as age and sex, underlying diseases including diabetes, hypertension, hyperlipidemia, cardiovascular and respiratory disorders were assessed. Clinical presentations among the two groups were compared. Hematological and biochemical tests were performed at baseline, day 3, and 7. We also followed the patients for one month, no side effects or complains was recorded. Blood oxygen saturation, Immunoglobulin M (IgM) and IgG antibodies level, isolation time, and time to return to work were also assessed in

each group.

The primary endpoints were the effects of honey bee products in addition to antiviral treatment on COVID-19 patients, and changes in laboratory findings were secondary endpoints.

Statistical analysis

For statistical analysis, statistical package for the social sciences (SPSS) version 22.0 was used. The Shapiro-Wilk and Kolmogorov-Smirnov tests were performed to evaluate the normal distribution of quantitative data. The baseline characteristics were compared, using the Chi-square test, t-student, or Mann-Whitney U test, as appropriate. Repeated measures analysis of variance (ANOVA) was performed for the data collected at multiple time points. Quantitative data are presented as mean ± SD, and qualitative variables are expressed as percentages. The significance level was set at $P < 0.05$ (95% Confidence Interval (CI)). The trend of changes during hospitalization was assessed using Microsoft Excel 2019.

Results

Among a total of 70 individuals who were screened for eligibility between April and September 2020, 50 patients were randomly assigned to receive honey pollen, royal jelly, and propolis plus their standard treatment (24 patients, intervention group) or receive standard medical treatment only (26 patients, control group). Twenty patients were excluded from the study. Before the beginning, two patients were excluded because of loss of consciousness and one, because of gastrointestinal bleeding. Three patients had a history of allergic reactions to honey and warfarin. The rest of them refused to sign a written informed consent.

In this study, a total of 50 patients were enrolled. 24 patients were assigned to the intervention group. The mean age in the intervention and control groups was 45.30 ± 15.70 and 53.70 ± 13.30 years, respectively. The sex distribution between the two groups was 65.20% female in the intervention group and 36.40% in the control group ($p > 0.05$). The prevalence of symptoms and underlying diseases (Table 1) among the two studied groups showed no difference ($p > 0.05$).

In this study, evaluation of the effects of royal jelly, propolis and bee pollen on the COVID-19 disease course showed a significant effect on the functional class and COVID-19 patient symptoms. the mean oxyyen saturation and fatigue were also improved significantly in the intervention grup (Figure 1).

Baseline laboratory data including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lymphocyte count, interleukin-6, D-Dimer, biochemical and hematological parameters including fasting blood sugar, blood urea nitrogen, creatinine, liver enzymes, alkaline phosphatase, and total bilirubin showed no significant differences among the two groups (Table 2 and Figure 2). The duration of hospitalization in the intervention and control groups was 8.18 ± 3.64 days and 8.45 ± 3.90 days, respectively, which was not statistically different ($p = 0.20$). The mean oxygen saturation in the intervention group was 92.70 ± 2.10 and in the control group was 89.20 ± 7.10. The mean changes of oxygen saturation in the intervention group were 2.03 ± 5

and in the control group was 1.61 ± 5 (p = 0.80). We found a significant antibody production in the intervention group after 1 week. In the intervention group, six patients had positive antibodies after supplementation treatment; while only one patient in the control group had positive antibodies after one week (p < 0.05).

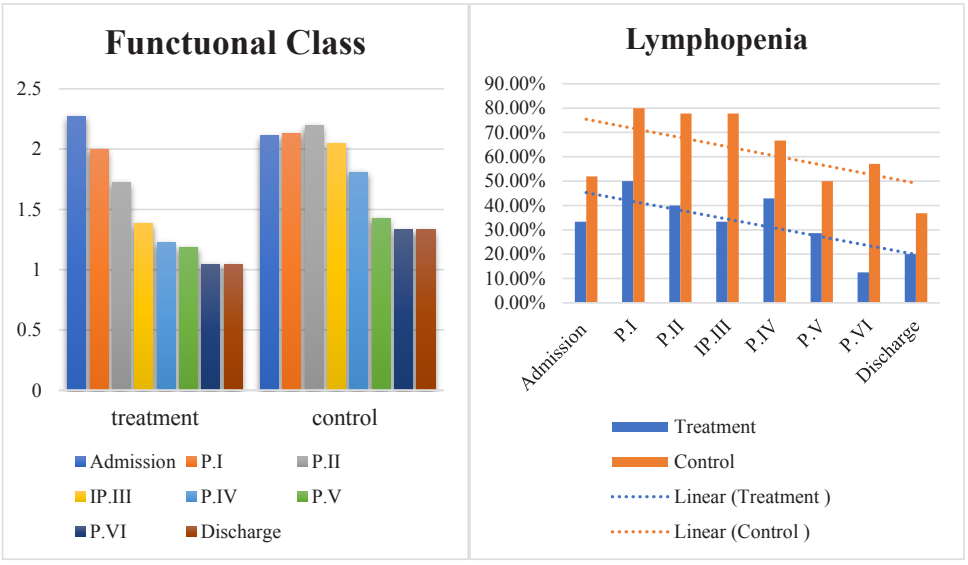
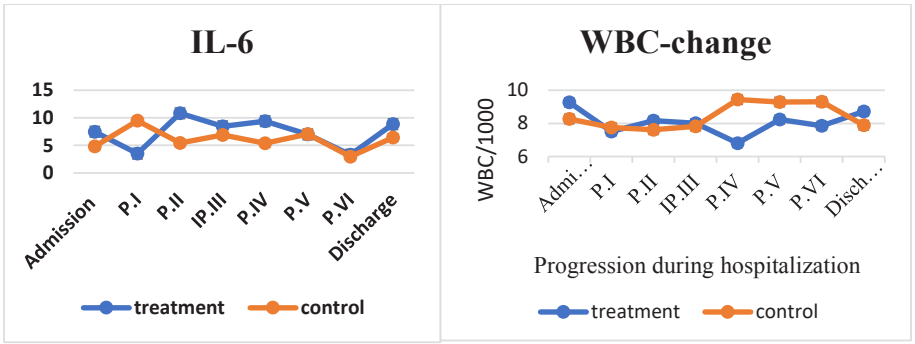


Figure 1. Improvement of functional class (a) and lymphopenia (b) in intervention and control group. (P1 refer to day one after start of the supplement.)



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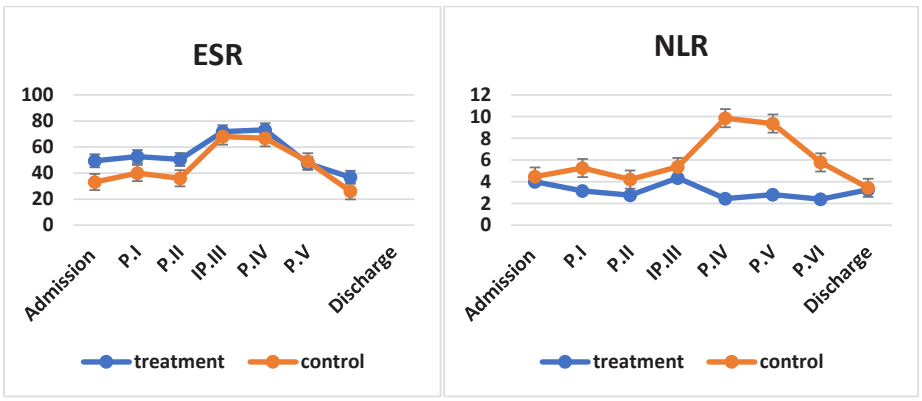

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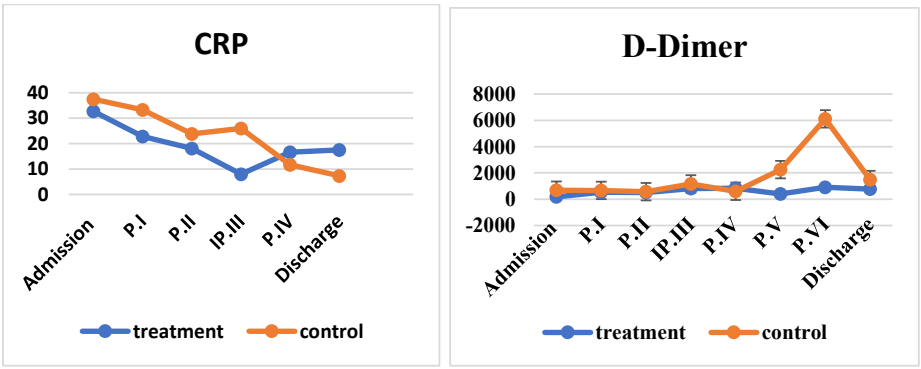

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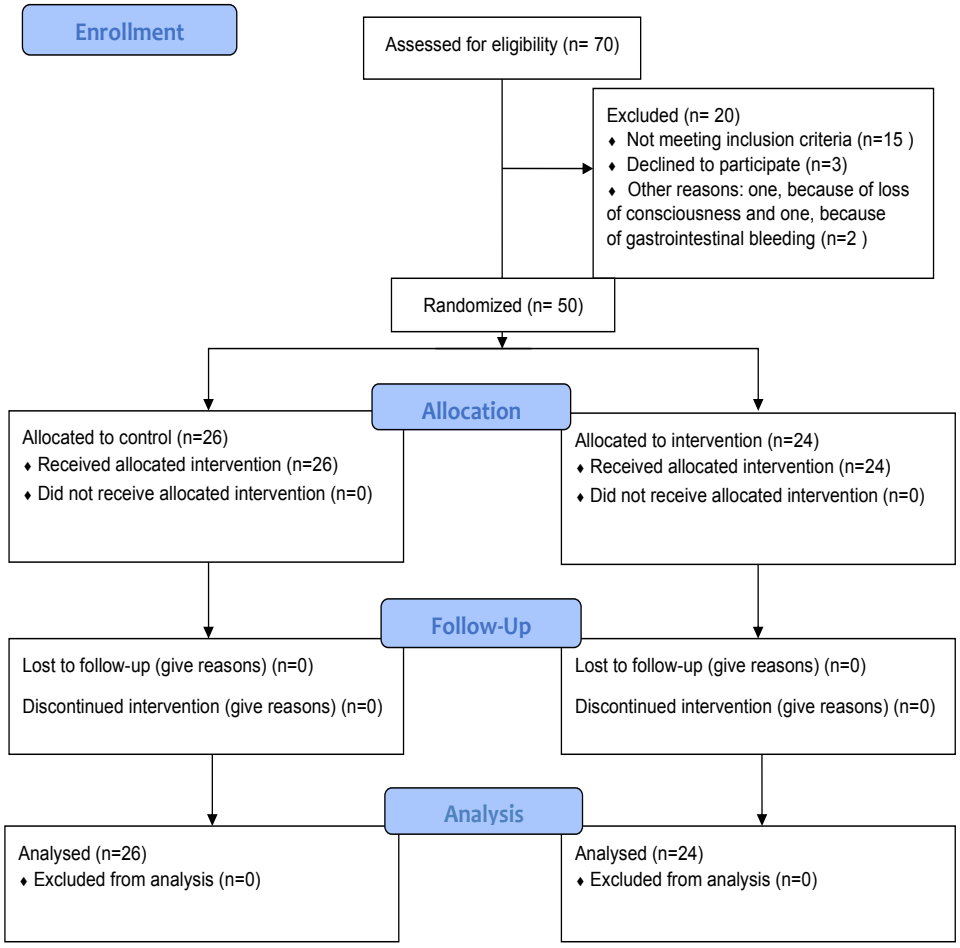
Figure 2. Trend of laboratory tests including interleukin 6 (IL-6) (a), WBC, ESR, NLR, CRP, D-Dimer from admission to discharge.



CONSORT diagram of N-chromosome royal jelly, propolis, and bee pollen supplementation in COVID-19 patients

Table1. Comparison of the baseline characteristics between treatment and control groups

	Intervention	Control	P value
Age (year)	45.3±15.70	53.7±13.3	0.08
Sex (female%) Functional class Symptoms	65.2 2.2±0.7	36.4 2.1±0.5	0.9 0.46
Cough %	17(70.8%)	20(76.9%)	0.8
Sputum %	9 (37.5%)	10 (38.5%)	0.8
Dyspnea%	19 (79.1%)	22 (84.6%)	0.6
Myalgia%	11 (45.8%)	7 (26.9%)	0.7
Diarrhea%	5 (20.8%)	6 (23.1%)	0.13
Vomiting%	3 (12.5%)	1 (3.8%0)	0.96
Fever%	10 (41.6%)	13 (50%)	0.91
Chilling Fever% Underlying Diseases	9 (37.5%)	10 (38.5%)	0.2
DM %	3 (12.5%)	7(28%)	0.2
HTN%	9 (37.5%)	7 (28%)	0.41
Cardiovascular Diseases%	5 (20.8%)	6 (24%)	0.6
Respiratory Diseases	5(20.8%)	2(8%)	0.17
Hypercholesterolemia	6 (25%)	3 (12%)	0.21

Table 2. Comparison of the baseline laboratory data between treatment and control groups

	Intervention	Control	P value
O2-Sat%	90.18±4.6	88.42±7.5	0.349
WBC/1000	9.2±4.8	8.2±5.13	0.5
IL-6	7.4±5.10	4.8±0.70	0.536
D-Dimer	180±91.4	687.±619.9	0.051
FBS	128.55±59.651	157.17±91.2	0.364
BUN	33.7±19.9	34.3±14.6	0.904
Creatinine	0.95±0.342	1.04±0.4	0.461
AST	30.63±14.4	35.32±16.5	0.331
ALT	31.7±27.30	40.56±26.2	0.287
ALK.P	193.5±51.294	183.54±89.1	0.674
Total Bilirubin	0.78±0.39	0.8±0.37	0.798
ESR	49.41±36.7	33.13±34.3	0.154
CRP	32.7±22.3	37.5±24.3	0.602

Discussion

In the present study we evaluated the effects of royal jelly, propolis, and bee pollen on the COVID-19 disease course which showed a significant effect on the functional class based on New York Heart Association Functional Classification (NYHA) and COVID-19 patient symptoms. The mean oxygen saturation, and fatigue were also improved significantly in the intervention group.

Royal jelly inhibits the production of some pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and IL-1 and limits capillary permeability in the acute phase of inflammation, leading to lower inflammatory response in the body [14]. The antiviral activity of this product has not been proved yet, so further studies are needed in this regard [14]. Propolis can inhibit virus proliferation with the effects on the virus genome, inhibition of viral polymerase and binding to the viral nucleic acid. So a reduction in viral multiplication and even a virucidal action will be expected with this product. Flavonoids as a category of major components of bee products has demonstrated suppression of the pro-inflammatory activities of cyclooxygenase-2 (COX–2) and inducible nitric oxide synthase (iNOS) [15]. Recent studies revealed that in severely ill patients, reduced numbers of the cluster of differentiation 4^+ ($CD4^+$) T cells, $CD8^+$ T cells, B cells, and natural killer cells (macrophage) and elevated serum levels of pro-inflammatory cytokines including TNF-α, IL-6, IL-2, and some other interleukins, characterized as cytokine storm have observed. Also, CRP and D-dimer are found to be abnormally high [12]. Although a well-coordinated immune system is a key element in defense against this infection, a dysregulated immunity due to uncontrolled cytokine release will result in excessive inflammation and body organ damages [2]. Thus high virus titers and subsequent hyperinflammatory responses will lead to high morbidity and mortality observed during pathogenic COVID-19 infection [13].

A retrospective Egyptian study described the use of oral TaibUVID -a combination of *Nigella sativa* L., *Matricaria chamomilla* L. and natural honey- as an adjuvant or primary therapy in COVID-19 patients, as well as a prophylactic agent in individuals in contact with confirmed COVID-19 patients (doctors and family members). *N. sativa* and *Anthemis hyalina* DC. exhibited a plethora of health and immunity-promoting activities. Experimentally, these herbs, especially chamomile, efficiently lowered the survival of SARS-CoV in infected culture cells [17].

Some randomized controlled clinical trials (RCTs) assessed the effectiveness of bee products in patients with a confirmed diagnosis of COVID-19. A Brazilian RCT reported that treating hospitalized COVID-19 patients with a single oral daily dose of Brazilian green propolis (EPP-AF®) was associated with significant reductions in the length of hospital stay and renal injury. Propolis treatment was not associated with a decrease in the need for oxygen therapy, unlike our study [18].

In this study, although all patients showed improvement in their symptoms, oxygen saturation, functional class, these findings were found to be

more significant in bee product users. About inflammatory parameters, there were no significant differences between the two groups.

Limitations

The present study was limited by its small number of cases in single-center and noncritical ill patients. Therefore, larger, multicenter studies are needed to further confirm the clinical utility of bee products even in critically ill situations.

Conclusion

In the context of correlation between the pathogenicity of cytokine storm and dysregulated body immune response caused by coronavirus with disease severity, it seems that inhibition of excessive production of pro-inflammatory cytokines and modulation of body immune response is an effective treatment option. The results revealed that the use of royal jelly, propolis, and bee pollen had not a curative effect in COVID-19 patients, but they minimized the disease symptoms. So honey bee products can be considered as an adjuvant to COVID-19 therapy and can be continued in a longer duration (up to one or two months) to better assess the degree of an immune response. It is recommended to use another form of bee products instead of oral consumption, especially in gastrointestinal involvement, because patients with nausea and vomiting can hardly tolerate oral medicines. Moreover, in critically ill patients that are connected to a ventilator, inhaled forms of royal jelly and propolis may be useful.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved retrospectively by the Ethics Committee of the National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences IR.SBMU.NRITLD.REC.1399.048). Prospectivity registered IRCT number is IRCT20200209046427N1.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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