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Original Article

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The Effect of Hesperidin on Laboratory Parameters of Patients with COVID 19: A Preliminary Report of a Clinical Trial Study

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Abstract

Background: Hesperidin is a secondary metabolite of the flavonoid group. Due to its antioxidant, anti-inflammatory and anti-viral properties, it can be helpful as a treatment option for patients with COVID 19.

Methods: This study was conducted as a clinical trial in Masih Daneshvari Hospital in Tehran. After providing complete explanations and obtaining written consent, patients with new coronavirus (COVID-19) were included in the study if they met the inclusion criteria. 20 patients with the new coronavirus (COVID-19) were included in the study. Patients were then randomly divided into hesperidin and control groups. Patients in the hesperidin group received 1 *mg* of hesperidin orally intravenously every 6 hours for 5 days, whereas in the control group, they did not. Then, the desired variables were measured during the research period. Patients were monitored for adverse drug reactions based on clinical symptoms and signs. The results were evaluated with regard to the design of the questionnaire and its completion using t-test and SPSS16 software.

Results: Patients with equal gender ratio were studied and diabetes mellitus with a prevalence of 60% had the highest prevalence among patients. On the other hand, 85% of these patients presented with bilateral lung involvement. Using hesperidin decreased lymphocytes, CRP, ESR, LDH, D-dimer, and IL-6 and increased WBC, Hb and Plt. None of the mentioned changes were significant ($p>0.05$).

Conclusion: Utilizing hesperidin could not cause significant changes in the level of immunological and inflammatory factors in patients with COVID 19.

Keywords: COVID19, Hesperidin, Flavonoid, Anti-inflammatory, Immunological factors

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus that is contagious in humans. It mainly enters human cells by binding to the receptor Angiotensin Converting Enzyme 2 (ACE2) (1). SARS-CoV-2 spike homotrimer focusing upon one protein subunit with an ACE2 binding domain highlighted SARS-CoV-2 spike homotrimer with one protein subunit highlighted (2).

Binding RBD with ACE2 is critical for SARS-CoV-2 entrance to the cell and features a crucial role in membrane fusion of the virus (3). Without proper receptor binding, there's no more S protein conformational change, thus viral entry to cells will decrease. Hesperidin can target the binding between hACE2 and RBD region of Spike protein of Sars-CoV-2 by filling the shallow pit of RBD (4). A hydrogen bond is formed between Tyr440 of S protein and the Oxygen atom of hesperidin (5). Hesperidin can hamper the contamination of cells expressing ACE2 receptor by preventing fusion. Therefore, the immune system will have more time to fight the virus (6).

These cases can clearly reveal the modulatory properties of hesperidin on the immune system of patients (7). Since the balance of stable conditions of the patient's body undergoes drastic changes in an infectious disease, establishing a stable stability in immunophysiological conditions of this group of patients is the most significant step for their recovery (8). Thus, hesperidin is expected to play an effective role in this regard.

One of the properties that makes hesperidin suitable for the treatment of SARS-COV-2 is its antiviral properties (9). Dong *et al* stated that hesperidin enhanced cell-autonomous immunity by modulating MAP kinase signaling pathways via up-regulating p38 and JNK activation while down-regulating ERK activation (10). Q-PCR assay showed that antiviral state-associated expression genes in the infected A549 cells, including RANTES, IP10, MCP1, IFNa, IFNb, IFNc, *etc.*, were significantly increased by hesperidin treatment (11-14). Hesperidin treatment increased the expression of the antiviral gene in infected A549 cells (12,15). On the other hand, it further improved the activation of p38 and JNK signaling pathways. Hesperidin treatment led an increase in both p38 and phosphorylated p38 proteins (12,13). These results were also confirmed in a report published by Hajialyani *et al* (16). Hespertin and its metabolites suppress LPS-induced phosphorylation of JNK1 / 2 and p38 by LPS and activate NF-KB, iNOS gene expression and cyclooxygenase (COX)-2 in RAW264.7 cells (16). Ma-Lauer *et al* stated that p53 inhibits replication of infectious SARS-CoV as well as replicons and human coronavirus NL63 (17). These results suggest that hesperidin, as a stimulatory factor in p53, may alter the condition toward impaired cell proliferation.

Materials and Methods

This study is in the form of a preliminary report of a clinical trial (IRCT20150725023332N5) which has been approved by the Ethics Committee in Biomedical Research of Masih Daneshvari Hospital in Tehran (IR.SBMU.NRITLD.REC.1399.126) and is currently being implemented. Patients with new coronavirus (COVID-19) were included in the study if they met the inclusion criteria after providing complete explanations and obtaining written consent (Figure 1). Adult (≥18 years old) had a positive RT-PCR of the throat swab for COVID-19 and severe disease. Respiratory rate ≥30 (breaths/min) or an SaO_2≤90% in room air or a partial pressure of arterial oxygen to percentage of inspired oxygen ratio (PaO_2/FiO_2) of ≤300 was considered as a moderate-to-severe disease. We excluded the patients with a history of other causes of lower respiratory infection such as viruses, bacteria, and fungal. Under the supervision of a physician,

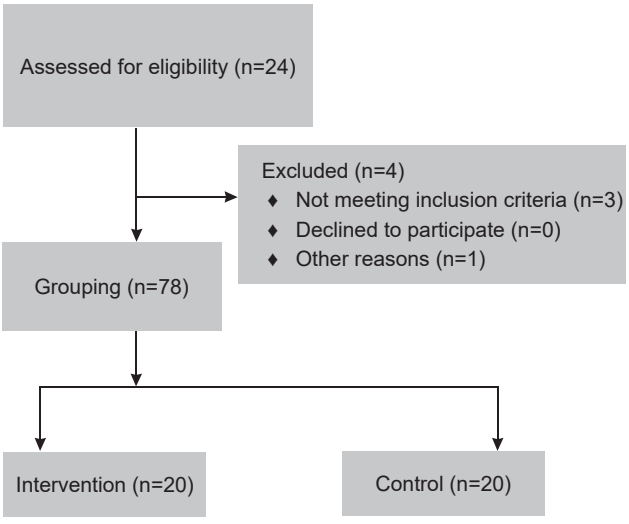

Figure 1. Consort chart of study.

Table 1. Background information of patients in the two groups of Intervention (treated with hesperidin) and control

ITEM	Intervention N (%)	Control N (%)	Total	p-value
Gender (Male)	5 (50%)	5 (50%)	10(50%)	1.000
HTN	7 (70%)	3 (30%)	10 (50%)	0.061
DM	8 (80%)	4 (40%)	12 (60%)	0.050
IHD	4 (40%)	2 (20%)	6 (30%)	0.076
Lung involvement				
Right	1 (10%)	1(10%)	2 (10%)	1.000
Left	1 (10%)	0 (0%)	1(5%)	0.090
Bilateral	8 (80%)	9 (90%)	17 (85%)	0.194
Lung involvement>50%	8 (80%)	7 (70%)	15 (75%)	0.120
GGO	10 (100%)	9 (90%)	19 (95%)	0.185
Consolidation	9 (90%)	7 (70%)	16 (80%)	0.107
Crazy paving	7 (70%)	5 (50%)	12 (60%)	0.083
Honey combing	5 (5%)	4 (40%)	9 (45%)	0.100

HTN: Hypertension; DM: Diabetes mellitus; IHD: ischemic heart disease; GGO: Ground-glass opacification

Table 2. Comparison of changes in laboratory factors in the two groups of Intervention (treated with hesperidin) and control

ITEM	Intervention (M± SD)	Control (M± SD)	p-value
RBC (10^{12}/*L*)	4.47±0.7	4.136±0.9	0.361
WBC (10^{9}/*L*)	10.46±4.7	8.03±4.1	0.234
Neutrophil (%)	74.08±16.08	72.1±10.8	0.758
Lymphocyte (%)	17.68±10.3	21.39±9.2	0.406
Monocyte (%)	4.75±7.5	2.31±3.2	0.354
Eosinophil (%)	0.52±0.7	0.05±0.1	0.054
Mix (%)	4.75±4.1	3.9±4.0	0.726
Hb (*g/mL*)	12.28±1.8	11.67±2.6	0.546
Hct (%)	38.04±5.0	35.89±6.0	0.394
Plt (×1000/*ml*)	221.9±84.4	195.2±115.8	0.562
MCV (*fL*)	86.16±8.8	89.11±12.3	0.544
MCH (*pg*)	27.51±3.2	30±5.3	0.219
MCHC (*g/mL*)	32.26±1.2	33.53±1.6	0.054
RDW	14.84±2.0	14.35±2.1	0.601
CRP (*mg/l*)	25.7±25.0	32.1±20.7	0.540
ESR (*mm/hr*)	40.77±22.1	66.4±40.9	0.113
LDH (*U/L*)	747.6±202.3	791.1±420.7	0.771
Ferritin (*U/L*)	867.37±614.3	535.42±438.7	0.256
CPK (*U/L*)	252.3±469.3	192.5±85.1	0.696
D-Dimer (*µg/mL*)	1891.5±2179.7	3861.66±2520.6	0.256
IL-6 (*U/L*)	9.55±10.0	12.29±6.4	0.482

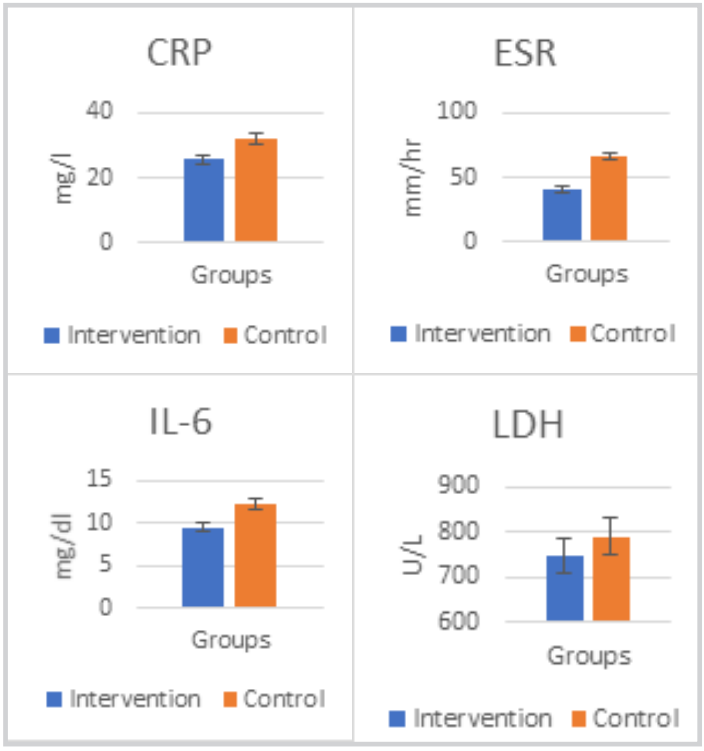

Figure 2. Comparison of mean inflammatory factors in patients in the study groups.

supportive treatment was provided if needed. Molecular detection of COVID-19 was confirmed by positive reverse transcriptase-polymerase chain reaction (RT-PCR) for coronavirus by extracting nucleic acid from samples by QiaSymphony system (QIAGEN, Hilden, Germany). Corman *et al* has described the process of detecting coronavirus using primer and probe sequences for screening and conformation (18). The research physicians are blind to the patient group and the patients are blind to the prescribed drug (double-blind). At this stage, 20 patients with the new coronavirus (COVID-19) were included in the study. Patients were then randomly divided into hesperidin and control groups. Patients in the hesperidin group received 1 *mg* of hesperidin (from Sichuan YuanHongFu Technology co) orally intravenously every 6 hours for 5 days, whereas in the control group, they did not. Then, the desired variables were measured during the research period. Patients were monitored for adverse drug reactions based on clinical symptoms and signs. The results were evaluated according to the design of the questionnaire and its completion using t-test and SPSS16 software [SPSS Inc, Chicago, IL, USA].

Results

Based on the results in table 1, patients with equal gender ratio were studied and diabetes mellitus with a prevalence of 60% had the highest prevalence among patients. On the other hand, 85% of these patients presented with bilateral lung involvement.

Although using hesperidin decreased lymphocytes, CRP, ESR, LDH, D-dimer, and IL-6 and increased WBC, Hb and Plt, none of these changes were significant ($p<0.05$) (Table 2).

Discussion

Hesperidin is one of the substances whose anti-inflammatory properties have been repeatedly evaluated (19). This plant secondary metabolite reduces NF-κB, AP-1 and SP-1 activation in lungs exposed to lipopolysaccharide and in A549 cells exposed to proinflammatory cytokine (17,20). Hesperidin, meanwhile, induces the expression of PPAR-γ and Bcl-2, balancing inflammatory responses and antioxidant changes in acute myocardial infarction in a rat model (21). Several other studies have shown that hesperidin treatment increased flow-mediated

dilation and reduced concentrations of circulating inflammatory biomarkers [high-sensitivity C-Reactive Protein (CRP), serum amyloid A protein, and soluble E-selectin)] (22).

Examination of serum levels of interleukins in patients infected with SARS -COV-2 has shown that many of these immunological factors are elevated (23). Factors such as IL-4, IL-6, IL-8 and IL-10 are among the indicators that are directly related to the severity of the disease and show increasing values (24). However, according to several other reports in various studies IL-2, IL-4, IL-5, IL-12 (in high dose), IL-13 and IL-17 has been shown to be decreased by hesperidin treatment (25-27).

Based on the results of the present study, what is clearly observed is the effect of hesperidin on the level of inflammatory factors. Although the changes were not statistically significant, it was shown that the utilizing hesperidin reduced these indicators (Figure 2). Earlier, Lorzadeh *et al* reported that taking hesperidin for 4 weeks had reduced serum CRP levels (28). Kawaguchi determined the asn before and after administration of hesperidin. Of 26 sets of determinations, ESR was retarded in 19, unchanged in three, and accelerated in four cases (29). Robbins administered hesperidin to 39 patients; 34 showed a decreased, two an unchanged, and three an increased ESR (30). Thus, by the usual interpretation that a high ESR reflects pathology, hesperidin in various cases showed beneficial, bad, or no effects. However, the data from the above experiments indicate that under certain conditions hesperidin can reverse its effect on blood cell aggregation. On the other hand, previous studies have also confirmed a decrease in LDH activity in hesperidin treatment against abnormal cell growth by altering protected membrane permeability or affecting cell growth.

The effect of hesperidin on the level of immune cells has been proven in many previous studies (31). The increase in the number of white blood cells in clinical and laboratory samples on the one hand and the effect of this substance on the level of cytokines on the other hand indicates the effect of hesperidin on immunological factors. However, according to the results of our study, changes in the control group were not significant ($p<0.05$).

The low statistical population on the one hand and the implementation of treatment in the form of research on the other hand can be considered as a reason for the lack of significant changes in these indicators. It seems that increasing the number of patients, increasing the dose of the drug or the duration of screening of patients with this drug, creates different conditions and shows the effect of hesperidin on COVID-19 patients.

Conclusion

Despite the changes in the use of hesperidin in improving the laboratory parameters of patients with COVID 19, these changes were not statistically significant.

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