IMMUNOREGULATION

2024. Vol 7

Research Paper

Chamomile Extracts Regulate IL-6 in PBMCs of Severe COVID-19 Patients

Rasoul Rashidi[1], Ensieh Sadat Mirsharif[1], Azadeh Rashidi[1], Tayebeh Radjabian[1], Tooba Ghazanfari[1*]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran.

Use your device to scan and read the article online

Citation Rashidi R, Mirsharif ES, Rashidi A, Radjabian T, Ghazanfari T. Chamomile Extracts Regulate IL-6 in PBMCs of Severe COVID-19 Patients. Immunoregulation. 2024; 7:E6. http://dx.doi.org/10.32598/Immunoregulation.7.6

http://dx.doi.org/10.32598/Immunoregulation.7.6

Article info:
Received: 10 Mar 2024
Accepted: 25 Apr 2024
Available Online: 10 May 2024

Keywords:
Chamomile, COVID-19, Peripheral blood mononuclear cells, Interleukin-6

ABSTRACT

Background: *Matricaria chamomilla* L. is advantageous due to its anti-inflammatory properties. The present study aimed to assess the efficacy of treating peripheral blood mononuclear cells (PBMCs) of COVID-19 patients with chamomile aqueous and ethanolic extracts on regulating interleukin-6 (IL-6) production.

Materials and Methods: PBMCs obtained from three COVID-19 volunteers were exposed to different concentrations (200, 300, 400, and 500 µg/mL) of ethanolic extract and (400, 500, 600, and 700 µg/mL) of aqueous extract, as well as 1 µg/mL prednisolone (positive control). The chemical content of the extracts was analyzed using high-performance liquid chromatography (HPLC). The production level of IL-6 was quantified using ELISA.

Results: The aqueous extract contained 0.26 and 0.11 mg/g of apigenin and quercetin, respectively, while the ethanolic extract contained 1.32 and 3.22 mg/g of apigenin and quercetin, respectively. Our data revealed that the ethanolic extract (all concentrations, including 200, 300, 400, and 500 µg/mL), aqueous extract (concentrations of 500, 600, and 700 µg/mL), and prednisolone (1 µg/mL) significantly lowered IL-6 production in PBMCs of COVID-19 patients ($P<0.05$). In terms of viability, 400 µg/mL of aqueous extract, 200 µg/mL and 300 µg/mL of ethanolic extracts, and 1 µg/mL of prednisolone had no significant effect on cell viability ($P>0.05$).

Conclusion: Given these results, the therapeutic potential of chamomile in hyper-inflammatory contexts can be related to its anti-inflammatory properties; however, its safety seems dose-dependent. Additional investigations are required to examine this herb's potential as a complementary treatment for inflammatory diseases.

*** Corresponding Author:**
Tooba Ghazanfari, PhD.
Address: *Immunoregulation Research Center, Shahed University, Tehran, Iran.*
Phone: *+98 (21) 66418216*
E-mail: *tghazanfari@yahoo.com; ghazanfari@shahed.ac.ir*

Introduction

The COVID-19 pandemic, which was first documented in December 2019, resulted in a substantial rise in the occurrence of diseases and deaths globally. The cause of this outbreak was linked to infection by the severe acute respiratory syndrome coronavirus (SARS-CoV)-2 virus. SARS-CoV-1 and SARS-CoV-2 are phylogenetically linked and are classified under the beta coronavirus genus [1]. While approximately 80% of SARS-CoV-2 confirmed cases are classified as asymptomatic or moderate, the remaining 20% of individuals may encounter severe symptoms that have the potential to be life-threatening [2]. Patients do not exhibit pronounced clinical signs during the early stages of disease progression. However, as the disease progresses, multiple organ failure and acute respiratory distress syndrome (ARDS) become evident. A significant proportion (86%) of mortality attributed to SARS-CoV-2 infection has been documented to result from respiratory failure [3].

The intricate immunopathogenicity of COVID-19 is linked to the virulence of the SARS-CoV-2 virus and the inability of the innate and adaptive immune systems to coordinate [4-6]. Additional putative routes that may contribute to immune responses against this virus include superantigens, autoimmunity, and pre-existing SARS-CoV immunity [7]. Patients with COVID-19 may have a dysregulated host immunological response, leading to the development of a potentially lethal inflammatory status referred to as "cytokine release syndrome" [8]. This scenario pertains to an unregulated inflammatory reaction, wherein there is an immediate and widespread secretion of pro-inflammatory cytokines in reaction to infectious stimuli. It has been noted that individuals requiring admission to the intensive care unit (ICU) exhibit an uncontrolled pro-inflammatory response [9]. ARDS is a significant contributor to mortality in patients with COVID-19. The substantial cause of this condition is an exaggerated immune response called a cytokine storm. The dysfunction of the respiratory epithelium in individuals with ARDS is associated with the involvement of various interleukins, including interleukin-1 (IL-1), IL-6, IL-17, and tumor necrosis factor-α, which play a fundamental role in the advancement of lung damage [10].

Throughout evolution and human history, natural products played a pivotal role in treating numerous ailments [11]. The COVID-19 pandemic has substantially impacted the application of herbal medicine. Nevertheless, a significant variation was observed in the utilization of herbal medications, necessitating further investigation to assess their efficacy and establish a comprehensive database documenting the herbal ingredients employed and their possible advantages and drawbacks [12]. Given the prevailing focus of global health on immunizing individuals who are in good health and treating those who are already ill, it is imperative to emphasize the significance of research endeavors to identify plant species with medicinal potential against different viral infections. Chamomile is widely recognized as a prominent herbal remedy employed in treating influenza or influenza-like symptoms owing to its antiviral properties [13]. This plant is significant in traditional Iranian contexts. Persian scholars, including Dioscoridus, Galen, Avicenna, Jarjani, and Ibn Bitar highlighted the therapeutic benefits of this plant on the neurological, digestive, and respiratory systems [14]. Chamomile contains notable biologically active compounds, such as apigenin, luteolin, patoletin, quercetin, and glucosides. Extensive research has substantiated the antimicrobial, antifungal, anti-inflammatory, antiallergic, wound-healing, antipyretic, antispasmodic [15, 16], sedative [17], anticancer [18], and antioxidant [19] properties of these substances.

In light of the significance of identifying medications that possess minimal adverse effects and are economically valuable for the worldwide healthcare system, this research endeavor was undertaken to assess the potential advantageous impacts of ethanolic and aqueous extracts obtained from chamomile on the concentration of pro-inflammatory cytokine production, IL-6, in peripheral blood mononuclear cells (PBMCs) of individuals afflicted with COVID-19.

Materials and Methods

Herbal materials

Preparation of chamomile aqueous and ethanolic extracts

Dried aerial portions of chamomile were acquired from the Pakan Bazar Isfahan firm in Iran. A total of 10.0 g of dried and ground material was macerated in either distilled water (100 mL) or 70% ethanol (100 mL) to prepare aqueous or ethanolic extracts, respectively. The systems were subjected to a three-day immersion period at ambient temperature, during which intermittent agitation was applied. Subsequently, the extracts were filtered using folded gauze and Whatman filters. Subsequently, the filtrate was concentrated using a rotary vacuum evaporator (temperature: 40 ˚C), forming a waxy substance. Finally, the substance was dried at a temperature of 37 ˚C. The samples were stored in the dark at 4 ˚C until testing.

For cell culture experiments, the desiccated substance obtained from the ethanolic and aqueous extracts was carefully weighed and subsequently dissolved in RPMI 1640 culture medium, which was sourced from Gibco (United States). Prednisolone was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. The prepared solution was combined with the culture medium to obtain the desired concentration. Notably, the medium's final concentration of DMSO did not exceed 0.5% [16, 20]. It should be noted that all materials under study were endotoxin-free.

High-performance liquid chromatography (HPLC) analysis

The two flavonoids, apigenin, and quercetin, present in chamomile's aqueous and ethanolic extracts, were evaluated using liquid chromatography following appropriate preparation procedures. The measurements were conducted in triplicates. Reference standards of apigenin (product number 10798, purity >95.0%) and quercetin (product number Q4951, purity >95.0%) were procured (Sigma-Aldrich). All other substances used in the experiment were of high analytical quality. A Shimadzu (LC 20, Japan) HPLC system comprising an LC-10Avp pump, CTO-10A column oven, SPD-10A UV-DAD detector, CBM-10A interface, and LC-10 Workstation was used. A Eurospher 100-5 C18 column was employed with a particle size of 5 µm, length of 250 mm, and diameter of 4.6 mm. The mobile phases used in the experiment consisted of two components: (A) consisting of 0.1% (v/v) phosphoric acid dissolved in water, and (B), which was acetonitrile. The gradient consisted of the following proportions of solvent A (v/v) over certain time intervals: 0-90% A at 0-10 minutes, 90-30% A at 10-40 minutes, 30-10% A at 40-45 minutes, 10% A at 45-50 minutes, and 10-90% A at 50-60 minutes (total run time=60 min). The flow rate of the stream was 1.5 mL/min, the injection volume was 20 µL, and the wavelength utilized for detection was 350 nm. Phenolic chemical standards, including apigenin and quercetin, were dissolved in methanol to measure the amounts of apigenin and quercetin in chamomile›s ethanolic and aqueous extracts. The apex in HPLC was determined by comparing the retention times of apigenin and quercetin. The apigenin and quercetin contents in the extracts were determined by analyzing the peak areas of apigenin and quercetin reference standards used as points of standard measurement. The results are presented as milligrams per gram of extract [21, 22].

Human subjects

Since the present study was conducted in the first year of the COVID-19 pandemic and no cell line related to this disease was available, we collected blood samples from three COVID-19 patients, and their PBMCs were used as a representative for the COVID-19 cell line. PBMCs were collected from confirmed COVID-19 patients hospitalized at Nikan Hospital, Tehran Province, Iran (February 2022). COVID-19 was confirmed based on the World Health Organization guidance [21]. In this regard, patients with positive SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) and lung involvement on chest computed tomography scans were enrolled in this study. Patients were hospitalized in the intensive care unit (ICU) and had IL-6 levels in the ELISA test above the normal range. Blood samples were collected on the first day of hospitalization. The subjects had a mean age of 72.6 years, and their main clinical manifestations were fever, cough, weakness, and body bruising.

In vitro experiment on peripheral blood mononuclear cells (PBMCs)

PBMCs isolation

PBMCs were isolated using previously described methods [23], wherein heparinized human peripheral whole blood was used. Specifically, 10 mL of whole blood was obtained from three individuals diagnosed with severe COVID-19 by venipuncture. Each patient was considered a separate case. The collected blood samples were transferred to heparin tubes. PBMCs were isolated from whole blood using density gradient centrifugation with Lymphodex (manufactured by Inno-Train, Germany).

In detail, 3 mL of phosphate-buffered saline (PBS) was added to each blood sample and then slowly poured into two Falcon tubes containing 3 mL of Lymphodex. The sample was centrifuged at 400 g for 30 min at 25°, forming a white layer. The white layer containing PBMCs was separated using a micropipette and poured onto 10 mL of PBS for cell washing. For this purpose, the solvent was centrifuged at 6° for 10 min at 300 g. In the next step, after draining the supernatant, the cells were re-washed by adding 5 mL of PBS to the cell pellet and centrifugation at 250 g for 10 min. Next, the supernatant was extracted, and cells were placed in RPMI medium enriched with 10% FBS (BIO-IDIA, Iran) and 1% penicillin-streptomycin solution (5000 units/mL penicillin; 5 mg/mL streptomycin) (Sigma Aldrich, Germany). Cell viability evaluation using trypan blue revealed that the separated cells exhibited a viability rate exceeding 98%.

PBMCs culture and experimental design

The PBMC's density was adjusted to 1×10^5 cells/well. Cell suspensions were distributed in 96-well plates containing RPMI medium with 10% FBS and 1% penicillin-streptomycin.

In our pilot study, we initiated treatment with very low doses of chamomile extract and gradually increased the concentration until we observed a noticeable decrease in IL-6 production. We then expanded our range to higher doses to assess the dose-response relationship. However, at higher concentrations, we observed a decreased cell viability, which prompted us to select the dose range used in the final study. This range allowed us to evaluate the anti-inflammatory effects of chamomile extracts while minimizing potential cytotoxicity. Accordingly, different concentrations of chamomile aqueous (400, 500, 600, and 700 µg/mL) and ethanolic (200, 300, 400, and 500 µg/mL) extracts were added to the cells for analysis. An equivalent number of wells was used for prednisolone as a positive control (1 µg/mL, purity 98%, Sigma Aldrich, Germany) instead of the chamomile extracts. The plates were placed in an incubator and kept at 37 ˚C for 24 hours in 5% CO_2 humidified air [23, 24].

Interleukin-6 (IL-6) cytokine secretion

The supernatants derived from the cell culture for subsequent ELISA analysis were stored at -70 ˚C. IL-6 levels were measured using commercially available kits (R&D, USA). After thawing at room temperature, the cell culture supernatants were transferred to the corresponding wells of a 96-well plate. All the reagents included in the kit were placed at room temperature and mixed carefully to avoid foaming. Each sample group was analyzed in replicate for each cytokine. A spectrophotometer (BioTek, USA) was used to measure the optical density of each well, and the absorbance values were recorded employing a microplate reader at a specific wavelength of 450 nm [23].

Statistical analysis

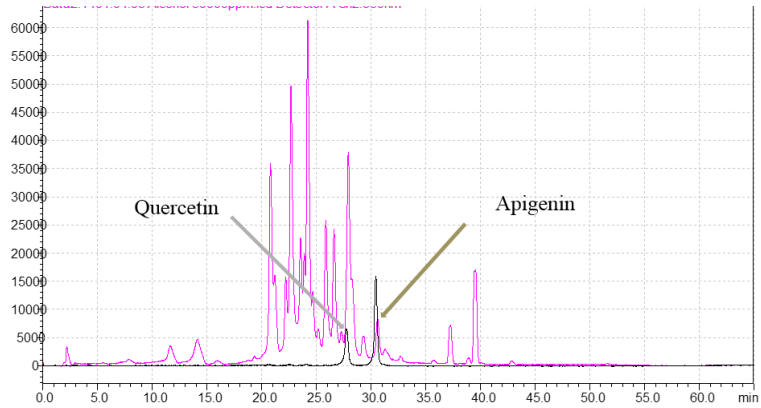
The results were expressed as the Mean±SE. Statistical analysis was conducted using SPSS software, version 23. Initially, we used the Shapiro-Wilk test to assess normality across all groups. The results indicated that most groups showed normal distribution with $P>0.05$. The statistical analysis employed in this study to measure the significance of differences between treatment groups involved using analysis of variance (ANOVA), followed by Duncan's multiple tests. Graphs were created using GraphPad Prism software, version 6.0. Statistical significance was set at $P<0.05$.

Results

HPLC analysis of the extracts

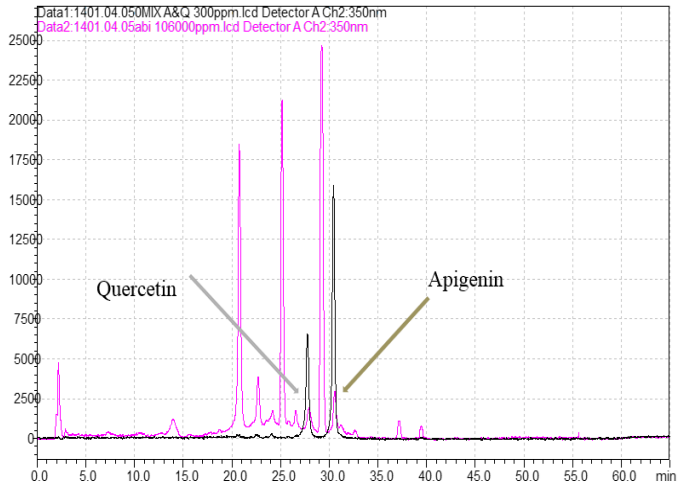
Figures 1 and 2 show the chromatographic profiles of the ethanolic and aqueous extracts of chamomile, respectively. The results of the quantitative study on phenolic compounds revealed the presence of the standard components, apigenin and quercetin, in both aqueous and ethanolic extracts. Figure 3 shows the chromatograms of the combination, including standard markers [22].

The contents of the aforementioned components in the ethanolic and aqueous extracts were ascertained based on the calibration curve of apigenin $y=448.073x–3568.48$ ($R^2=0.9959$) and quercetin $y=958.599x–11529.5$ ($R^2=0.9825$), where y represented the Summit region, and x denoted the concentration of analytes (50, 100,



IMMUNOREGULATION

Figure 1. The HPLC chromatogram of the ethanolic extract obtained from Chamomile, measured at a wavelength of 350 nm
HPLC: High-performance liquid chromatography.



IMMUNOREGULATION

Figure 2. The HPLC chromatogram of the aqueous extract obtained from Chamomile, measured at a Wavelength of 350 nm HPLC: High-performance liquid chromatography.

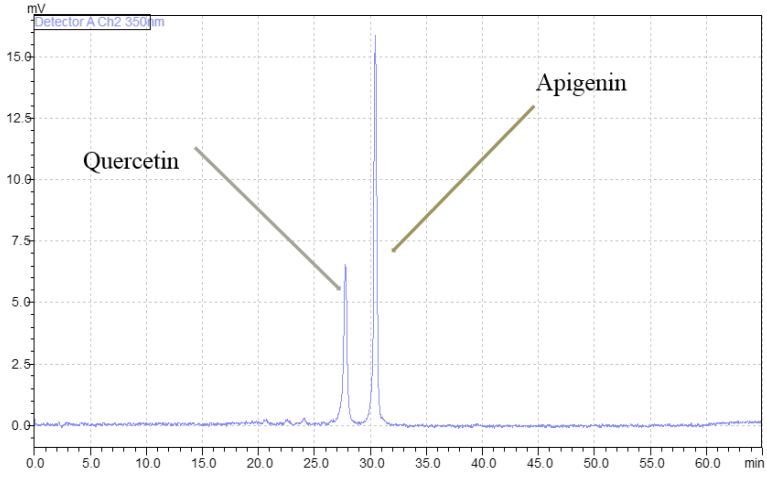
200, 300, and 400 µg/mL). Apigenin and quercetin were quantified in various extracts using calibration curves [22]. The amounts of apigenin and quercetin in the aqueous extract were 0.26 and 0.11, and in the ethanolic extract, they were 1.32 and 3.22 mg/g of dry chamomile powder, respectively.

IL-6 cytokine release after PBMCs treatment with Chamomile extracts

The production of IL-6 cytokine following the treatment of PBMCs with ethanolic and aqueous chamomile extracts was examined. The results indicated that PBMCs treated with the ethanolic extract at all concentrations, as well as the positive control group treated with prednisolone, exhibited significantly lower IL-6 production than the untreated group (P<0.001) (Figure 4). Furthermore, PBMCs exposed to the aqueous extract at concentrations of 500, 600, and 700 µg/mL, as well as the positive control group treated with prednisolone (1 µg/mL), exhibited a statistically significant decline in the production of IL-6 compared to the untreated group (P<0.01) (Figure 5). Compared to prednisolone (1 µg/mL), 300, 400, and 500 µg/mL of ethanolic extracts and 400, 500, and 700 µg/mL of aqueous extract significantly decreased IL-6 concentration.

The dose-response curve (Figure 6) shows that IL-6 production decreased with increasing doses of chamomile aqueous and ethanolic extracts.

It should be noted that the viability of the PBMCs was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) test in the first step



IMMUNOREGULATION

Figure 3. Apigenin and quercetin standard curve [22]

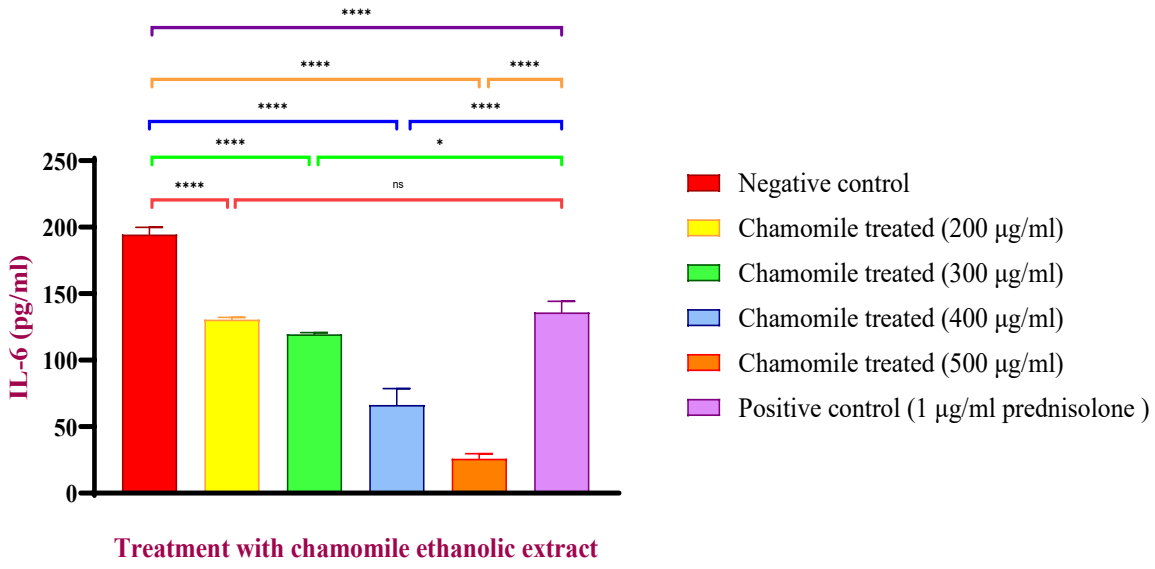


Figure 4. Effect of investigated concentrations of Chamomile ethanolic extract on IL-6 levels

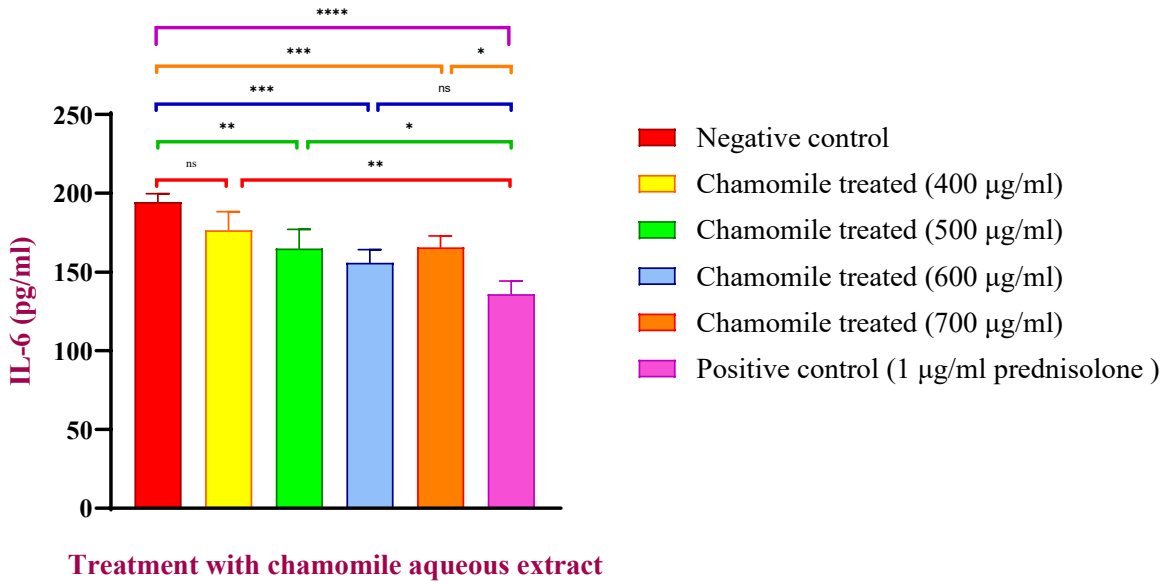
IMMUNOREGULATION

IL-6: Interleukin-6.

of the study. The result demonstrated that 400 µg/mL of aqueous extract, 200 µg/mL, and 300 µg/mL of ethanolic extract, as well as 1 µg/mL of prednisolone, did not cause a substantial decline in cell viability when compared to untreated cells [25].

PBMCs obtained from COVID-19 patients were treated with rising concentrations (200, 300, 400, and 500 µg/mL) of chamomile ethanolic extract and prednisolone (1 µg/mL). The concentration of IL-6 was measured in the cell culture medium of PBMCs treated with chamomile ethanolic extract for 24 h. Data are presented as Mean±SEM from three separate experiments; significant differences were determined using one-way ANOVA followed by Dunnett's post-hoc test ($^{*}P<0.05$ compared to untreated cells).

PBMCs obtained from COVID-19 patients were treated with rising concentrations (400, 500, 600, 700 µg/mL) of chamomile aqueous extract and prednisolone (1 µg/mL).



IMMUNOREGULATION

Figure 5. Effect of investigated concentrations of chamomile aqueous extract on IL-6 production

IL-6: Interleukin-6.

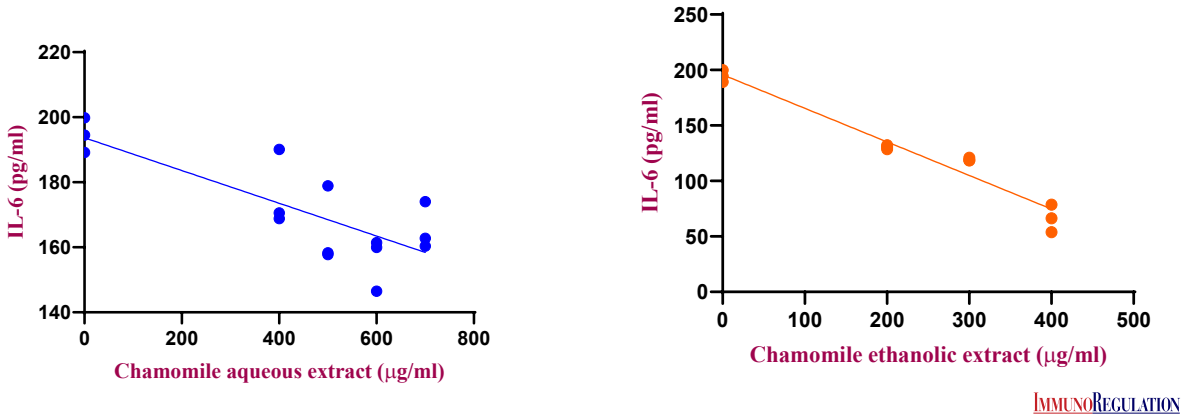


Figure 6. Dose-response curve related to IL-6 production in PBMCs cultured from COVID-19 patients under different concentrations of Chamomile aqueous and ethanolic extracts

IL-6: Interleukin-6; PBMCs: Peripheral blood mononuclear cells.

The concentration of IL-6 was measured in the cell culture medium of PBMCs treated with chamomile aqueous extract for 24 h. Data are presented as Mean±SEM from three separate experiments; significant differences were determined using one-way ANOVA followed by Dunnett's post-hoc test ($P<0.05$ compared to untreated cells).

Discussion

The results of various studies demonstrate that chamomile possesses widely recognized properties that reduce inflammation and counteract the effects of oxidants. Furthermore, the anti-inflammatory effects have been demonstrated to result from inhibiting nitric oxide (NO) production [26]. The present study represents a novel examination of the potential anti-inflammatory properties of chamomile in hyperinflammation linked to COVID-19. Our results demonstrated the in vitro anti-inflammatory efficacy of aerial parts of chamomile extracts and, importantly, provided initial evidence suggesting that the observed immune-regulating effects of chamomile can be attributed to the modulation of immune cell function. This particular herb possesses properties that make it suitable for future and more extended research for incorporation into a complementary pharmaceutical product, as it has demonstrated a significant ability to reduce inflammation in a manner equivalent to the corticosteroid prednisolone, which has been approved for such purposes.

Previous studies have documented a notable disparity in IL-6 concentrations between individuals with severe and non-severe COVID-19 [27]. Consistent with other studies suggesting the anti-inflammatory properties of different chamomile extracts and its metabolites, this study also demonstrated that chamomile effectively reduced inflammation by reducing the production of IL-6 by PBMCs. It is worth mentioning that in our first part of the study, the extracts exhibited minimal cytotoxicity towards cell viability when used at low concentrations (specifically, 400 µg/mL of aqueous extract and also 200 µg/mL and 300 µg/mL of ethanolic extract) [25] (Appendix 1). These results indicate that these extracts can be employed as anti-inflammatory immunomodulators. Consistent with these findings, De Cicco et al. examined the in vitro impact of chamomile extracts on lymphocyte proliferation, viability, and stimulation of proinflammatory cytokine levels in PBMCs. The study revealed that chamomile extract effectively suppressed the production of IL-6 by inhibiting the expression of genes associated with this cytokine. It highlighted the utilization of chamomile extract as an effective anti-inflammatory mediator. In the study by Cicco et al. the application of interferon gamma (IFN-γ) and lipopolysaccharides (LPS) to murine and human macrophages, in addition to a safe amount of chamomile essential oils (EOs), resulted in a substantial drop in the level of IL-6. This decrease was observed in cell supernatants and messenger ribonucleic acid (mRNA) levels [28]. The study conducted by Srivastava et al. demonstrated that the administration of chamomile effectively suppressed the secretion of prostaglandin $E_{(2)}$ caused by LPS in RAW 264.7 macrophages. The observed impact was determined to result from the suppression of cyclooxygenase-2 (COX-2) activity by chamomile. Furthermore, chamomile administration led to a significant downregulation of the expression of COX-2 mRNA and protein generated by LPS, while having no impact on the expression of COX-1 [29]. α-bisabol, often known as α-bis, has garnered pharmacological attention due to its presence in the essential oil derived from chamomile. The research carried

out by D'Almeida et al. demonstrated that the utilization of α-bis-lipid-core nano-capsules caused a significant drop in various indicators of lung inflammation and injury in a murine model of acute respiratory distress syndrome. These indicators included neutrophil infiltration, chemokine levels (particularly KC and MIP-2), airway hyperreactivity, myeloperoxidase activity, and lung tissue injury [30]. In support of these results, McKay et al. conducted research utilizing extracts derived from Matricaria recutita L. In this investigation, the primary components of the oil were identified as α-bis and its oxides. The researchers observed that the administration of these extracts effectively inhibited leukocyte infiltration, which was caused by concurrent carrageenan and prostaglandin E1 treatment in "Wistar" rats [31].

The difference in solubility between ethanol and water influences the extraction efficiency of bioactive compounds, potentially leading to higher concentrations in ethanolic extracts. According to Cowan [32], ethanol is highly efficient for extracting phenolic compounds. Our HPLC analysis showed that significantly higher amounts of flavonoids including apigenin and quercetin, were found in the ethanolic extract than in the aqueous extract. These compounds are known for their anti-inflammatory properties and may have contributed to the enhanced efficacy of the ethanolic extract observed in the present study. Apigenin and quercetin represent the principal phenolic anti-inflammatory compounds identified in chamomile [33]. Research results have indicated that apigenin has effectively reduced the proinflammatory enzymes' activity by suppressing the mRNA expression of COX and inducible nitric oxide synthase (iNOS) [34, 35]. Furthermore, previous studies have provided evidence of its ability to inhibit the release of IL-6 and IL-8, as well as its capacity to decrease the expression of adhesion molecules including ICAM-1, VCAM-1, and E-selectin, in response to cytokine stimulation [36]. The research conducted by Drummond et al. (2013) revealed that apigenin exhibited significant anti-inflammatory properties, as evidenced by its ability to decrease the levels of IL-6 by approximately 70%–90% at concentrations of 10 mM and higher [37]. Quercetin possesses the capacity to diminish the production of NO and TNF-a by human macrophages. Additionally, it has been noted that it diminishes the expression levels of COX-2 and inducible nitric oxide synthase (iNOS) mRNA [38]. Furthermore, Drummond et al. observed a notable decline in IL-6 levels following the quercetin administration [37].

Our results demonstrated that chamomile extracts, particularly ethanolic extracts, reduced excessive inflammation by suppressing IL-6 production in COVID-19 patients PBMCs. While additional research is required to ascertain the influence of various chamomile extracts on the modulation of additional inflammatory agents such as cytokines, chemokines, and immune cells involved in the pathology of COVID-19, the previously described discoveries also illuminate the immune-regulating role of chamomile extract in adaptive immunity. Furthermore, it is essential to conduct randomized, double-blind clinical trials involving human subjects to obtain clinically significant results about the potential of chamomile as an effective treatment for COVID-19. In addition to its recognized medical qualities, numerous investigations have highlighted concerns over the potentially harmful consequences of chamomile, particularly about allergic reactions that may occur after oral consumption [39] and contact dermatitis following topical applications [40, 41]. In addition, chamomile preparations contain many constituents, including chamazulene, cis-spiroether, and trans-spiroether. These constituents have demonstrated inhibitory effects on crucial drug-metabolizing enzymes in vitro, namely those relevant to human metabolism [42]. Therefore, it is imperative to consider the possibility of interactions between chamomile and different pharmaceutical substances.

Conclusion

This study demonstrates that aqueous and ethanolic extracts of Matricaria chamomilla L. effectively regulate IL-6 production in PBMCs from COVID-19 patients with severe manifestations. Notably, the safety profile of chamomile appears to be dose-dependent, indicating that careful consideration of dosage is crucial to maximize therapeutic benefits and minimize adverse effects. This study highlights the possible therapeutic potential of chamomile in reducing IL-6 production and opens avenues for future investigations into its role in broader inflammatory contexts.

This study has certain limitations. The small sample size restricted the generalizability of our findings. Additionally, the in vitro setup, although useful for initial exploration, does not fully capture the complex interactions in vivo. In addition, due to resource constraints during the study period, particularly given the extraordinary circumstances, it was not feasible to delve into the underlying mechanisms and more specific mediators within the scope of the current research. Future studies should explore the effect of chamomile extracts on key signaling pathways and cytokine production to provide deeper insights into their anti-inflammatory effects.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Shahed University, Tehran, Iran (Code: IR.SHAHED.REC.1400.129).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Planning the experiments: Tooba Ghazanfari, Tayebeh Radjabian and Rasoul Rashidi; carried the experiments: Rasoul Rashidi and Ensieh Sadat Mirsharif; Supervising the project: Tooba Ghazanfari and Tayebeh Radjabian; Data analysis: Rasoul Rashidi; Writing the original draft, review & editing: Rasoul Rashidi, Azadeh Rashidi, Tooba Ghazanfari and Tayebeh Radjabian; Data interpretation and final approval: All authors.

Conflicts of interest

The authors declared no conflict of interest.

References

[1] Zhang HP, Sun YL, Wang YF, Yazici D, Azkur D, Ogulur I, et al. Recent developments in the immunopathology of COVID-19. Allergy. 2023; 78(2):369-88. [DOI:10.1111/all.15593] [PMID]

[2] Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. Journal of Medical Virology. 2020; 92(4):424-32. [DOI:10.1002/jmv.25685] [PMID]

[3] Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Medicine. 2020; 46(5):846-8. [DOI:10.1007/s00134-020-05991-x] [PMID]

[4] Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020; 181(5):1036-45. e9. [DOI:10.1016/j.cell.2020.04.026] [PMID]

[5] Sokolowska M, Lukasik ZM, Agache I, Akdis CA, Akdis D, Akdis M, et al. Immunology of COVID-19: Mechanisms, clinical outcome, diagnostics, and perspectives-A report of the European Academy of Allergy and Clinical Immunology (EAACI). Allergy. 2020; 75(10):2445-76. [DOI:10.1111/all.14462] [PMID]

[6] Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Brüggen MC, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy. 2020; 75(7):1564-81. [DOI:10.1111/all.14364] [PMID]

[7] Maggi E, Azzarone BG, Canonica GW, Moretta L. What we know and still ignore on COVID-19 immune pathogenesis and a proposal based on the experience of allergic disorders. Allergy. 2022; 77(4):1114-28. [DOI:10.1111/all.15112] [PMID]

[8] Wang Y, Wang Y, Chen Y, Qin Q. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. Journal of Medical Virology. 2020; 92(6):568-76. [DOI:10.1002/jmv.25748] [PMID]

[9] Channappanavar R, Perlman S. Pathogenic human coronavirus infections: Causes and consequences of cytokine storm and immunopathology. Seminars in Immunopathology. 2017; 39(5):529-39. [DOI:10.1007/s00281-017-0629-x] [PMID]

[10] Montazersaheb S, Hosseiniyan Khatibi SM, Hejazi MS, Tarhriz V, Farjami A, Ghasemian Sorbeni F, et al. COVID-19 infection: an overview on cytokine storm and related interventions. Virology Journal. 2022; 19(1):92. [DOI:10.1186/s12985-022-01814-1] [PMID]

[11] Denaro M, Smeriglio A, Barreca D, De Francesco C, Occhiuto C, Milano G, et al. Antiviral activity of plants and their isolated bioactive compounds: An update. Phytotherapy Research. 2020; 34(4):742-68. [DOI:10.1002/ptr.6575] [PMID]

[12] Frost R, Heinrich M, Pendry B, Bhamr S. COVID-19 and herbal medicine: A practitioner survey. European Journal of Integrative Medicine. 2021; 48:101974. [DOI:10.1016/j.eujim.2021.101974]

[13] Habibzadeh S, Zohalinezhad ME. Antiviral activity of Matricaria chamomilla in the treatment of COVID-19: Molecular Docking study. European Journal of Integrative Medicine. 2021; 48:101975. [DOI:10.1016/j.eujim.2021.101975]

[14] Ghaffari F, Moein E. [The chamomile in islamic and iranian traditional medicine context (Persian)]. Journal of Islamic and Iranian Traditional Medicine. 2013; 4(1):79-85. [Link]

[15] Chauhan ES, Jaya A. Chamomile an ancient aromatic plant-A review. Journal of Ayurveda Medical Sciences. 2017; 2(4):251-5. [DOI:10.5530/jams.2017.2.26]

[16] Srivastava JK, Gupta S. Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. Molecular and Cellular Pharmacology. 2009; 1(3):138. [DOI:10.4255/mcpharmacol.09.18] [PMID]

[17] Mehmood MH, Munir S, Khalid UA, Asrar M, Gilani AH. Antidiarrhoeal, antisecretory and antispasmodic activities of Matricaria chamomilla are mediated predominantly through K(+)-channels activation. BMC Complementary and Alternative Medicine. 2015; 15:75. [DOI:10.1186/s12906-015-0595-6] [PMID]

[18] Al-Dabbagh B, Elhaty IA, Elhaw M, Murali C, Al Mansoori A, Awad B, et al. Antioxidant and anticancer activities of chamomile (Matricaria recutita L.). BMC Research Notes. 2019; 12(1):3. [DOI:10.1186/s13104-018-3960-y] [PMID]

[19] Srivastava JK, Shankar E, Gupta S. Chamomile: A herbal medicine of the past with bright future. Molecular Medicine Reports. 2010; 3(6):895-901. [DOI:10.3892/mmr.2010.377] [PMID]

[20] Molnar M, Mendešević N, Šubarić D, Banjari I, Jokić S. Comparison of various techniques for the extraction of umbelliferone and herniarin in Matricaria chamomilla processing fractions. Chemistry Central Journal. 2017; 11(1):78. [DOI:10.1186/s13065-017-0308-y] [PMID]

[21] Dong X, Lan W, Yin X, Yang C, Wang W, Ni J. Simultaneous determination and pharmacokinetic study of quercetin, luteolin, and apigenin in rat plasma after oral administration of Matricaria chamomilla L. extract by HPLC-UV. Evidence-Based Complementary and Alternative Medicine. 2017; 2017:8370584. [DOI:10.1155/2017/8370584] [PMID]

[22] Tabet A, Boukhari A, Nouidjem Y. Phenolic content, HPLC analysis and antioxidant activity extract from Tamarix articulata. Journal of Advanced Pharmacy Education & Research. 2018; 8(4). [Link]

[23] Chen R, Curran J, Pu F, Zhuola Z, Bayon Y, Hunt JA. In vitro response of human peripheral blood mononuclear cells (PBMC) to collagen films treated with cold plasma. Polymers. 2017; 9(7):254. [DOI:10.3390/polym9070254] [PMID]

[24] Shin HY, Kuo YC, Hsu FL, Mao YW, Lee MH. Regulation of cytokine production by treating with Chinese tonic herbs in human peripheral blood mononuclear and human acute monocytic leukemia cells. Journal of Food and Drug Analysis. 2010; 18(6):1. [DOI:10.38212/2224-6614.2227]

[25] Rashidi R, Ghazanfari T, Radjabian T, Mirsharif ES. [The effect of aqueous and ethanolic extracts of chamomile on the vital activity of peripheral blood leukocytes of patients with Covid-19 (Persian)]. Daneshvar Medicine. 2023; 30(6):1-9. [DOI:10.22070/daneshmed.2023.17141.1304]

[26] Bhaskaran N, Shukla S, Srivastava JK, Gupta S. Chamomile: An anti-inflammatory agent inhibits inducible nitric oxide synthase expression by blocking RelA/p65 activity. International Journal of Molecular Medicine. 2010; 26(6):935-40. [DOI:10.3892/ijmm_00000545] [PMID]

[27] Halim C, Mirza AF, Sari MI. The association between TNF-α, IL-6, and vitamin D levels and COVID-19 severity and mortality: A systematic review and meta-analysis. Pathogens. 2022; 11(2):195. [DOI:10.3390/pathogens11020195] [PMID]

[28] De Cicco P, Ercolano G, Sirignano C, Rubino V, Rigano D, Ianaro A, et al. Chamomile essential oils exert anti-inflammatory effects involving human and murine macrophages: Evidence to support a therapeutic action. Journal of Ethnopharmacology. 2023; 311:116391. [DOI:10.1016/j.jep.2023.116391] [PMID]

[29] Srivastava JK, Pandey M, Gupta S. Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity. Life Sciences. 2009; 85(19-20):663-9. [DOI:10.1016/j.lfs.2009.09.007] [PMID]

[30] D'Almeida APL, Pacheco de Oliveira MT, de Souza ÉT, de Sá Coutinho D, Ciambarella BT, Gomes CR, et al. α-bisabolol-loaded lipid-core nanocapsules reduce lipopolysaccharide-induced pulmonary inflammation in mice. International Journal of Nanomedicine. 2017; 12:4479-91. [DOI:10.2147/IJN.S130798] [PMID]

[31] McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.). Phytotherapy Research. 2006; 20(7):519-30. [DOI:10.1002/ptr.1900] [PMID]

[32] Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999; 12(4):564-82. [DOI:10.1128/CMR.12.4.564] [PMID]

[33] Harbourne N, Jacquier JC, O'Riordan D. Optimisation of the extraction and processing conditions of chamomile (Matricaria chamomilla L.) for incorporation into a beverage. Food Chemistry. 2009; 115(1):15-9. [DOI:10.1016/j.foodchem.2008.11.044]

[34] Liang YC, Huang YT, Tsai SH, Lin-Shiau SY, Chen CF, Lin JK. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. Carcinogenesis. 1999; 20(10):1945-52. [DOI:10.1093/carcin/20.10.1945] [PMID]

[35] García-Mediavilla V, Crespo I, Collado PS, Esteller A, Sánchez-Campos S, Tuñón MJ, et al. The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. European Journal of Pharmacology. 2007; 557(2-3):221-9. [DOI:10.1016/j.ejphar.2006.11.014] [PMID]

[36] Gerritsen ME, Carley WW, Ranges GE, Shen CP, Phan SA, Ligon GF, et al. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. The American Journal of Pathology. 1995; 147(2):278-92. [PMID]

[37] Drummond EM, Harbourne N, Marete E, Martyn D, Jacquier J, O'Riordan D, et al. Inhibition of proinflammatory biomarkers in THP1 macrophages by polyphenols derived from chamomile, meadowsweet and willow bark. Phytotherapy Research. 2013; 27(4):588-94. [DOI:10.1002/ptr.4753] [PMID]

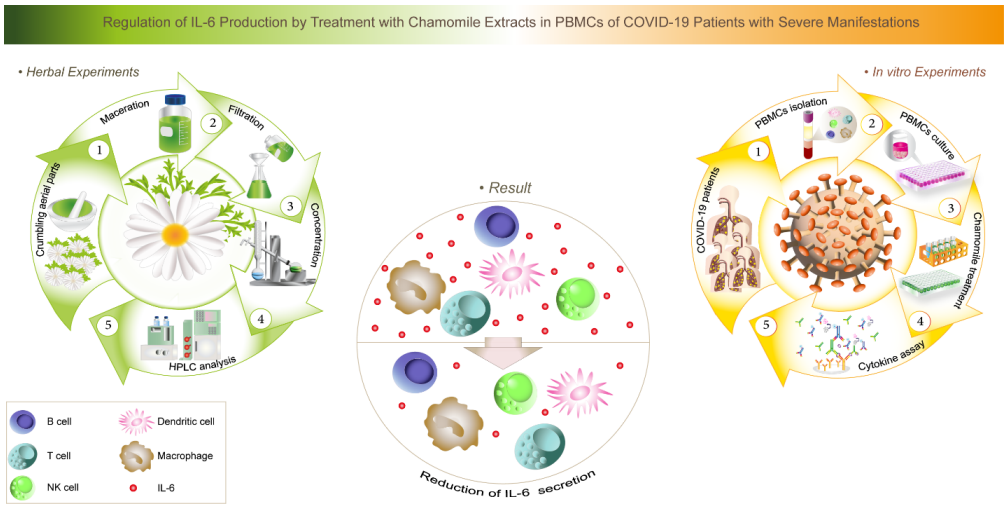
[38] Tordjman C, Coge F, Andre N, Rique H, Spedding M, Bonnet J. Characterisation of cyclooxygenase 1 and 2 expression in mouse resident peritoneal macrophages in vitro; interactions of non steroidal anti-inflammatory drugs with COX2. Biochimica et Biophysica Acta. 1995; 1256(2):249-56. [DOI:10.1016/0005-2760(95)00026-9]

[39] Paulsen E. Contact sensitization from Compositae-containing herbal remedies and cosmetics. Contact Dermatitis. 2002; 47(4):189-98. [DOI:10.1034/j.1600-0536.2002.470401.x] [PMID]

[40] Federici E, Multari G, Gallo FR, Palazzino G. [Herbal drugs: From traditional use to regulation (Italian)]. Annali Dell'Istituto Superiore di Sanita. 2005; 41(1):49-54. [PMID]

[41] De Smet PA. Herbal remedies. The New England Journal of Medicine. 2002; 347(25):2046-56. [DOI:10.1056/NEJMra020398] [PMID]

[42] Rodriguez-Fragoso L, Reyes-Esparza J, Burchiel SW, Herrera-Ruiz D, Torres E. Risks and benefits of commonly used herbal medicines in Mexico. Toxicology and Applied Pharmacology. 2008; 227(1):125-35. [DOI:10.1016/j.taap.2007.10.005] [PMID]



Appendix 1. Graphical abstract