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RESEARCH ARTICLE

Effect of n-Hexane Extract from *Tanacetum argenteum* (Lam.) Willd. subsp. *argenteum* on the Secretion of Proinflammatory Cytokines in THP-1 Cell Line

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SUMMARY

Inflammation is an initial biological process that involves the activation of the immune system in response to injury, infection or exposure to toxic agents. During this process, cytokines, small proteins produced by immune cells, play a vital role in regulating the immune response. Inflammatory cytokines, including interleukins, tumor necrosis factor-α, nitric oxide, and interferon-gamma, initiate the immune response and promote inflammation. Natural products are frequently a source of potential anti-inflammatory compounds, and screening natural products can lead to the discovery of novel bioactive compounds. The present study aimed to investigate the effects of n-hexane extract from Tanacetum argenteum subsp. argenteum on the lipopolysaccharide-induced inflammatory response in human macrophages THP-1 cell. Cells were incubated with different concentrations of n-hexane extract, and the inhibitor effects of the extract exposure on various cytokine secretions were determined. The findings demonstrated that n-hexane extract dramatically decreased the levels of interleukin-6, interleukin-1β, and tumor necrosis factor-α in differentiated THP-1 cells, indicating the remarkable anti-inflammatory potential of the extract. The n-hexane extract inhibited the secretion of interleukin-6 and interleukin-1β even at the lowest dose of 1 µg/ml. However, a significant reduction in tumor necrosis factor-α secretion was observed at 5 µg/ml and above concentrations. Importantly, the results of the study indicated that both the n-hexane extract and its active component, parthenolide, exhibit comparable effects. Furthermore, in silico analysis of toxicogenomic data revealed the interactions between the active component of the n-hexane extract and interleukin-6, interleukin-1β, and tumor necrosis factor.

Key Words: *Tanacetum argenteum subsp. argenteum, inflammatory response, human macrophage cells, toxicogenomic data analysis*

Tanacetum argenteum (Lam.) Willd subsp. argenteum n-Hekzan Ekstresinin THP-1 Hücre Hattında Proinflamatuar Sitokinlerin Salgılanması Üzerine Etkisi

ÖZ

İnflamasyon, infeksiyon, hasar ya da toksik ajanlara maruz kalmaya yanıt olarak immün sistemin aktivasyonunu içeren biyolojik süreçtir. Bu süreç sırasında, immün hücreleri tarafından üretilen küçük proteinler olan sitokinler, inflamatuar cevabın düzenlenmesinde hayati bir rol oynar. İnterlökinler, tümör nekroz faktörü-α, nitrik oksit ve interferon-gama gibi inflamatuar sitokinler immun yanıtı başlatır ve inflamasyonun indüklenmesine yol açar. Doğal ürünler sıklıkla anti-inflamatuar aktivite potansiyeli olan bileşiklerin kaynağı olup ve çalışmalar yeni biyoaktif bileşiklerin keşfine yol açabilir. Bu çalışma, Tanacetum argenteum subsp. argenteum'dan hazırlanan n-hekzan ekstresinin THP-1; insan makrofaj hücresinde lipopolisakkarit ile indüklenen inflamatuar yanıt üzerindeki etkilerini araştırmayı amaçlamıştır. Hücreler farklı konsantrasyonlarda n-hekzan ekstresi ile inkübe edilmiş ve hücrelerin ekstreye maruziyetinin çeşitli sitokinlerin sekresyonları üzerindeki inhibitör etkisi belirlenmiştir. Sonuçlar, n-hekzan ekstresinin farklılaştırılmış THP-1 hücrelerinde interlökin-6, interlökin-1β ve tümör nekroz faktörü-α düzeyini önemli ölçüde azalttığını ve ekstrenin anti-inflamatuar potansiyelini ortaya koymuştur. N-hekzan ekstresi, en düşük doz olan 1 µg/ml'da bile interlökin-6 ve interleukin-1β ekspresyonunu inhibe etmiştir. Ancak, interlökin-6 sekresyonunda 5 µg/ml ve üzeri konsantrasyonlarda anlamlı azalma gözlenmiştir. Daha da önemlisi, çalışmanın sonuçları n-hekzan ekstresi ile aktif bileşeni olan partenolidin karşılaştırılabilir etkiler sergilediğini göstermiştir. Ayrıca, toksikogenomik verilerin in silico analizi n-hekzan ekstresinin aktif bileşeni ile interlökin-6, interlökin-1β ve tümör nekroz faktörü arasındaki etkileşimleri ortaya koymuştur.

Anahtar Kelimeler: *Tanacetum argenteum subsp. argenteum, inflamatuar cevap, insan makrofaj hücreleri, toksikogenomik veri analizi*

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INTRODUCTION

The inflammation process is a coordinated response involving the activation of signaling pathways and aims to initiate tissue repair and protect the body from further damage (Chen et al., 2017). Inflammatory stimuli, including pathogens, toxins, or endogenous factors, can trigger signaling pathways in cells, stimulating the production of inflammatory mediators. Inflammatory cytokines bind to specific receptors on the surface of immune cells, including interleukin-1 receptor (IL-1R), toll-like receptors, tumor necrosis factor (TNF) receptor and IL-6 receptor (Dinarello et al., 2018; Parameswaran & Patial, 2010; Tanaka et al., 2014). Once activated, these receptors can initiate intracellular signaling pathways that stimulate the excessive production of pro-inflammatory cytokines and chemokines. These molecules recruit immune cells to the area of inflammation, enhance their activation and survival, and induce tissue damage or repair (Kany et al., 2019). The precise balance of these inflammatory responses is critical for protecting cells from infection or tissue injury and maintaining tissue homeostasis. However, dysregulated, excessive and chronic inflammation contributes to the development of various diseases, such as diabetes, rheumatoid arthritis, cancer and cardiovascular diseases (Chen et al., 2017). Therefore, the underlying mechanisms of inflammatory processes in multiple diseases and developing or identifying novel and effective inflammatory compounds are essential for the therapeutic strategies of numerous diseases (Furman et al., 2019).

Multiple investigations have been conducted to identify natural products, including herbal extracts and phytochemicals, that can act as anti-inflammatory agents and improve the therapeutic effect of synthetic drugs (Nisar et al., 2023). *Tanacetum argenteum (Lam.)* Willd. subsp. *argenteum* (Asteraceae) is an essential annual plant found exclusively in Inner and South Anatolia (Albayrak et al., 2017). Previous studies have shown that the subspecies of *T. argenteum* differ in their total flavonoid components and levels of parthenolide. *T. argenteum* subsp. *argenteum* has been found to have the highest levels of both parthenolide and total flavonoid among all the subspecies (Orhan et al., 2015). Therefore, it is essential to investigate the biological activities of *T. argenteum* subsp. *argenteum* and determine the potential therapeutic effects of all extracts and pure compounds. In our previous study, we demonstrated that the *n*-hexane extract of this plant exhibited significant activity in the iNOS assay, and we also conducted an MTT assay to determine the cytotoxicity potential of related plant extracts. *N*-hexane extract was found to exhibit potent cytotoxicity to PC-3 cells (Albayrak et al., 2017). However, the anti-inflammatory activity of this extract had not been examined in terms of possible suppression of the secretion of various cytokines.

In the present study, we aimed to investigate the potential anti-inflammatory effects of the *n*-hexane extract from *T. argenteum* subsp. *argenteum* on lipopolysaccharide (LPS)-treated THP-1 cells. LPS was utilized to induce inflammation and promote the secretion of inflammatory cytokines in cells. We incubated differentiated THP-1 cells with increasing concentrations of *n*-hexane extract following LPS stimulation to determine the effects of the extract on the levels of interleukin (IL)-6, IL-1β and tumor necrosis factor-α (TNF-*a*). Furthermore, we performed an *in silico* toxicogenomic data analysis to predict the compound responsible for suppressing cytokine secretion. In our previous publication, we confirmed the presence of the parthenolide compound in the *n*-hexane extract using thin layer chromatography (TLC) (Albayrak et al., 2017), and in the current study, we employed high performance liquid chromatography (HPLC) for the same purpose. Moreover, we conducted a toxicogenomic evaluation of its interaction with genes and proteins based on the parameters we measured experimentally.

MATERIAL AND METHODS

Cell Culture and Materials

THP-1 (ATTC, TIB-202, USA) human monocyte cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum. The cells were kept at 37°C under 5% CO_2 to maintain an appropriate environment for their survival. All experiments were conducted after the treatment of the monocytes with 25 μM of phorbol 12-myristate 13-acetate (PMA) for 24 hours. This treatment was applied to differentiate the monocytes into adherent macrophages used in the subsequent experiments. IL-6 (cat. no. ab46027), IL-1β (cat. no. ab214025) and TNF-α (cat. no. ab181421) assay kits were provided by Abcam (MA, U.S.A.). PMA, parthenolide, and all other chemicals were obtained from Sigma-Aldrich (Darmstadt, Germany).

Preparation and HPLC-UV Analysis of *N*-hexane Extract

The aerial parts of the plant were collected from Mount Nemrut, Adıyaman, in 2022, and the plant material was identified by Prof. Şura Baykan Ozturk. A voucher specimen was stored in the herbarium of the Faculty of Pharmacy of Ege University (IZEF 6029). The aerial parts of the plant were dried with a constant airflow in shadow. Then the dried material was powdered in a grinder to be ready for extraction. The plant parts (100 g) were extracted with *n*-hexane (200 mL) using an ultrasonic water bath 2 times, each for 6 hours. After filtration with filter paper, the obtained extract was evaporated to dryness with a rotary evaporator under low pressure at 40°C. The extract was kept at 4°C until the bioactivity experiments.

HPLC analysis was performed to determine whether the activity of *n*-hexane extract is due to the presence of parthenolide, a significant bioactive molecule of *T. argenteum*. The HPLC analyses were carried out in accordance with the previously described method (Avula et al., 2006). In brief, the solutions of parthenolide and extract were prepared in DMSO and diluted with mobile phase B. 500 ppm standard parthenolide solution and 200 ppm *n*-hexane extract were analyzed by employing the ACE5-C18 column (100 Å, 5 μm, 4.6 x 250 mm) with gradient elution of mobile phase A (water) and mobile phase B (acetonitrile: methanol (90:10, %v/v)). The flow rate was 1 ml/min, and the wavelength range detection was 210 nm.

Treatment of Cells with *N*-hexane Extract and Inflammation Induction by LPS

Differentiated THP-1 cells (pretreated with 25 μM PMA for 24 hours) were categorized into four groups: one group served as the control (medium without LPS and extract), another group was exposed to LPS, the third group was treated with parthenolide before LPS treatment, and the last group was treated with *n*-hexane extract before LPS treatment.

Stock solutions of parthenolide and *n*-hexane extract were prepared in DMSO. Following, appropriate amounts of cell medium were used to dilute stock solutions to desired final concentrations. The final concentration of DMSO did not exceed 1%. The cells were incubated with increasing concentrations of parthenolide (0-5 μg/ml) or *n*-hexane extract (0-100 μg/ml) for 2 hours. After the incubation period, the cells were then stimulated with the LPS (final concentration: 2μM) for 24 hours. The working final concentration of LPS was selected in accordance with the literature (Liu et al., 2018). 2 μM is the dose level of LPS that induces inflammation and the secretion of inflammatory cytokines without leading to cytotoxicity in the differentiated THP-1 cells. Moreover, the cytotoxicity of 2 μM LPS was also controlled in our laboratory via MTT assay, and no significant cytotoxic effect was seen (data not shown), similar to a previous study (Liu et al., 2018). Subsequently, the measurement of IL-6, IL-1β and TNF-α were conducted by commercial kits.

Measurement of IL-1β, IL-6 and TNF-α Levels

The levels of IL-1β (ab214025), IL-6 (ab46027) and TNF-α (ab181421) in the cell supernatant were mea-

sured using ELISA kits according to the manufacturer's instructions. After the incubation period, as detailed above, 40 µl of supernatants were collected and transferred to novel wells. Subsequently, the specific antibody cocktails of each ELISA kit were added to wells, and the mixture was incubated for 1 hour again at room temperature. Then, TMB solution was added, and following 10 minutes, absorbance was measured by operating a microplate reader at a wavelength of 450 nm (Zhang et al., 2020). Experiments were repeated three times and conducted in triplicate.

Identification of Shared Genes Associated with Both Parthenolide and Inflammatory Processes

We examined the Comparative Toxicogenomics Database data to investigate the potential correlation between parthenolide and inflammation (Davis et al., 2023). The MyVenn tool was utilized to identify shared genes associated with parthenolide and inflammation from a dataset retrieved on December 25, 2023 (Davis et al., 2023).

Investigating Interactions Among Genes and Proteins

GeneMANIA was employed to examine the network of interactions among genes, with a specific focus on *Homo sapiens* as the target organism (Franz et al., 2018). In the investigation of protein-protein interactions (PPI) linked to parthenolide and inflammation, the String v.12 database was utilized, setting a minimum required interaction score threshold of 0.9, and the organism chosen was *Homo sapiens* (Szklarczyk et al., 2023). The analysis included the utilization of Cytoscape version 3.10.1 (Shannon et al., 2003). Furthermore, to pinpoint the essential proteins contributing to inflammation, the Network Analyzer Cytoscape plugin was employed to evaluate betweenness (BC), closeness (CC), degree centralities (DC), and number of undirected edges.

Statistical Analysis

All data were expressed as mean ± standard error. Results were compared by one-way ANOVA analysis employing the GraphPad Prism. A statistically significant difference was considered when the p-value was less than 0.05%.

RESULTS AND DISCUSSION

Previous studies of *Tanacetum* species primarily focused on their chemical composition and potential therapeutic effects, including anti-inflammatory, anti-oxidant and anti-cancer properties (Jain et al., 1999; Rosselli et al., 2012; Vilhelmova et al., 2020). Nasri et al. reported that the anti-inflammatory effects were detected only in the alcoholic extract of *T. balsamita*, and quercetin is responsible for the observed effects (Nasri et al., 2014). Bukhari et al. studied several extracts of *T. artemisioides* and demonstrated that *n*-hexane extract had the highest anti-inflammatory potential. *T. argentum* subsp. *argenteum* is an endemic and essential plant (Bukhari et al., 2007). However, there is limited research on its biological and therapeutic potential. In our previous study, we evaluated the anti-inflammatory activity of *T. argenteum* subsp. *argenteum* employing NF-κB and iNOS assays, and we found that the *n*-hexane extract had higher anti-inflammatory potential than the others similar to *T. artemisioides* (Albayrak et al., 2017). Therefore, in this follow-up study, we investigated the possible inhibition of the *n*-hexane extract on the secretion of inflammatory cytokines in THP-1 cells.

First, we determined the presence of parthenolide in *n*-hexane extract by the HPLC-UV system. As shown in Figure 1, the blue chromatogram displays the retention time of the standard parthenolide molecule. The retention time of parthenolide (500 ppm) was 17.85 min. The red chromatogram indicates the primary molecular peak in the 200 ppm *n*-hexane extract. The retention time of the main peak was 17.9 min. Upon analyzing the two chromatograms, it was observed that the *n*-hexane extract predominantly comprised parthenolide (Figure 1).

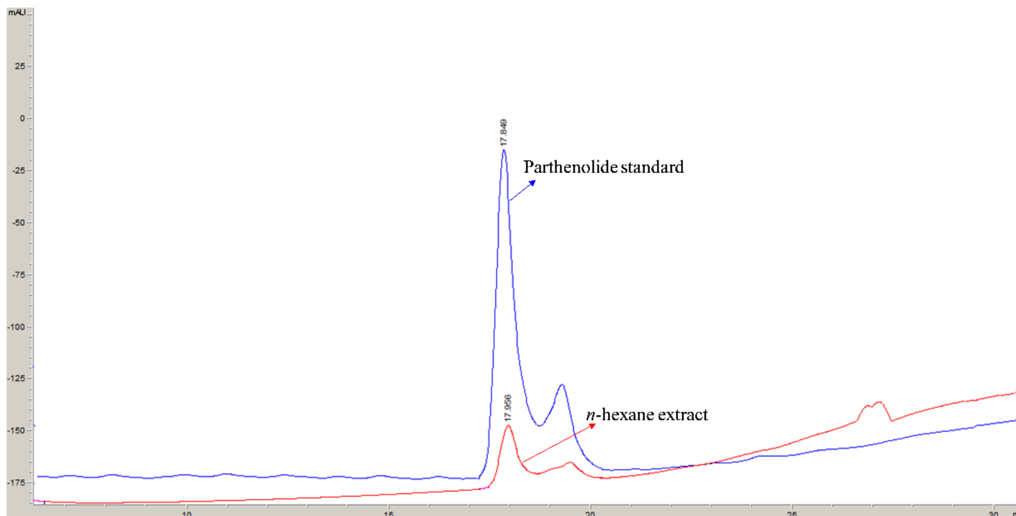

Figure 1. The HPLC-UV (210 nm) analysis. Blue chromatogram, parthenolide standard; red chromatogram, *n*-hexane extract.

Then, we exposed differentiated THP-1 cells to 2 µM LPS for 24 hours and detected IL-6, IL-1β, and TNF-α expression levels using ELISA. The final concentration level of LPS was determined as the dose value that triggers inflammation without causing cytotoxicity in the cell based on a previous study (Liu et al., 2018) and our laboratory experience. The incubation of differentiated THP-1 cells with 2 µg/mL LPS resulted in a significant increase in the levels of inflammatory cytokines, including IL-6, IL-1β, and TNF-α, compared to the control. Following LPS exposure, IL-6, IL-1β levels and TNF-α levels increased from 8.6±0.44 pg/ml to 31.2±0.57 pg/ml, 325.12±1.56 pg/ml to 570.3±1.75 pg/ml, and 180.33±1.18 pg/ml to 420.63±1.77 pg/ml, respectively, indicating the efficient induction of inflammation (Figure 2-4).

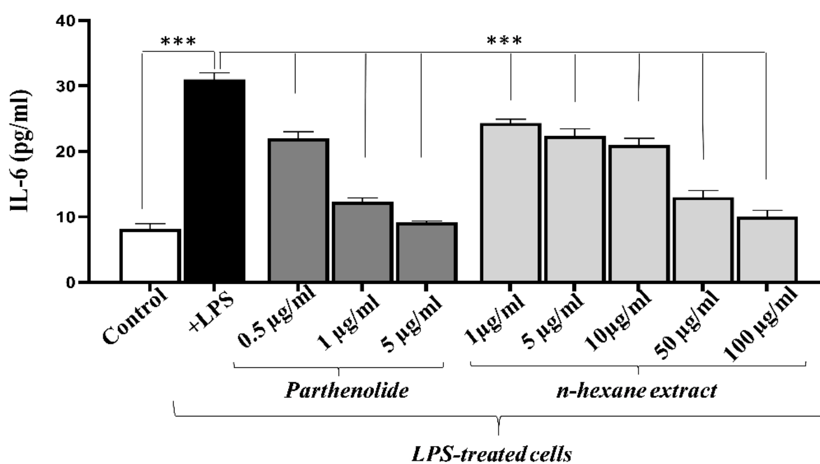

Figure 2. The effects of *n*-hexane extract on the levels of IL-6 in LPS-treated THP-1 cells. Cells were incubated with various concentrations of parthenolide (0.5-5 µg/ml) or *n*-hexane extract (1-100 µg/ml) for 24h. The control group comprised cells incubated solely in the medium, without LPS, parthenolide or *n*-hexane extract. *** $p < 0.0001$.

Then, the anti-inflammatory effects of increasing concentrations of *n*-hexane extract were investigated in the presence of excessive inflammation in THP-1 cells stimulated with LPS. In order to evaluate the anti-inflammatory potential of the extract, cells were also treated with parthenolide standard. As shown in Figure 2, IL-6 secretion was measured as 31.2±0.57 pg/ml in LPS-treated cells. Unsurprisingly, parthenolide exposure decreased IL-6 levels compared to cells incubated with LPS alone. Cells treated with 0.5, 1, and 5 µg/ml of parthenolide exhibited 22.4±0.57, 12.33±0.31, and 9.27±0.12 pg/ml IL-6 secretion, respectively. Furthermore, *n*-hexane extract markedly reduced the secretion of IL-6 compared to only LPS-treated cells; the inhibitory effect started at 1µg/ml and increased in a dose-dependent manner. Treatment with 1-100 µg/ml *n*-hexane extract resulted in 24.3±0.31-10.2±0.57 pg/ml IL-6 secretion in cells.

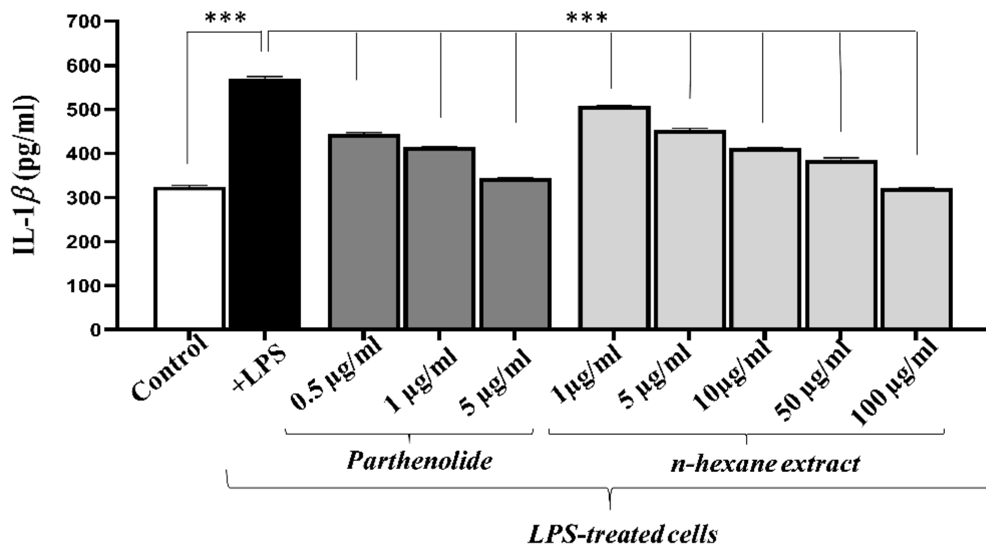

Figure 3. IL-1β levels of LPS-treated THP-1 cells after the incubation with the *n*-hexane extract or parthenolide. The cells representing the control group were incubated only in the medium, without the presence of LPS or extract. *** $p < 0.0001$.

Moreover, parthenolide *or n*-hexane extract treatment dramatically diminished the secretion of IL-1β and TNF-α in differentiated THP-1 cells, as illustrated in Figures 3-4. IL-1β levels were measured as 445.3±1.12 pg/ml, 414.02±0.94 pg/ml, and 344.5±0.84 pg/ml with the treatment of 0.5 µg/ml, 1 µg/ml, and 5 µg/ml of parthenolide, while the level was 570.3±1.75 pg/ml in cells treated only with LPS (Figure 3). In a similar trend, 0.5, 1, and 5 mg/ml parthenolide also decreased TNF-α levels from 420.63±1.77 pg/ml to 382.67±1.18pg/ml, 342.23±0.27 pg/ml, and 215.08±0.61 pg/ml in LPS-treated cells, respectively (Figure 4). At a concentration of 1 µg/ml, the *n*-hexane extract did not significantly alter the level of TNF-α, while a significant decrease in IL-1β levels was detected. 1 µg/ml *n*-hexane extract decreased IL-1β levels from 570.3±1.75 pg/ml to 507.9±0.41 pg/ml (Figure 3). Furthermore, the levels of IL-1β and TNF-α secretion were significantly reduced with higher doses of the extract; treatment with 5 µg/ml extract resulted in 353.16±0.82 pg/ml TNF-α and 453.64±1.15 pg/ml IL-1β secretion. At the highest dose studied (100 µg/ml), IL-1β and TNF-α levels were measured as 321.36±0.81 and 201.7±1.26 pg/ml, respectively (Figures 3-4).

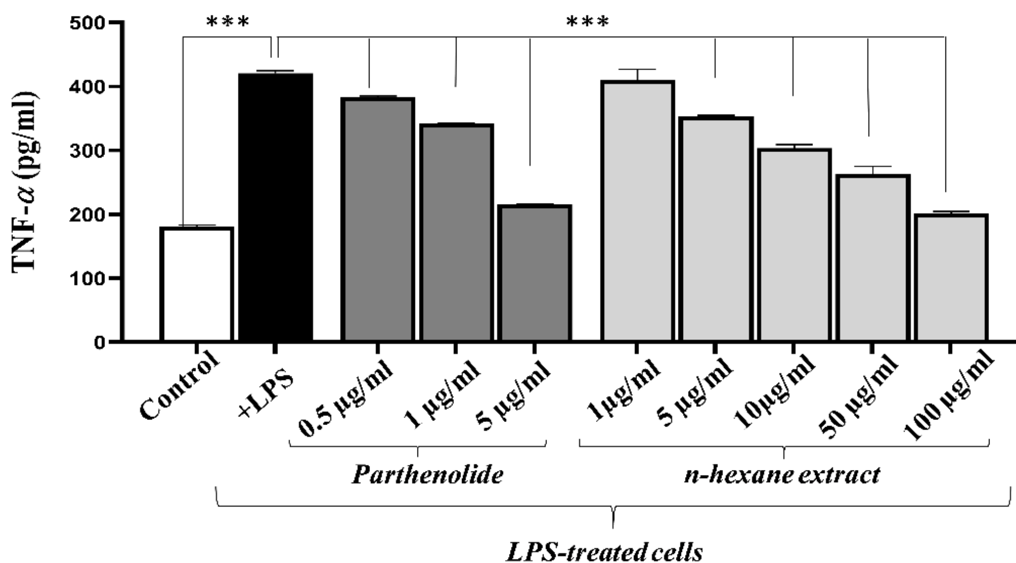

Figure 4. The effects of *n*-hexane extract and parthenolide on the levels of TNF-α in LPS-treated THP-1 cells. The control group comprised cells incubated solely in the medium, without LPS or extract. *** $p < 0.0001$.

The observed effect of *n*-hexane extract might be caused by apolar sesquiterpene lactones that contain γ-lactone (Gören et al., 2002). These lactones have multiple biological activities, including cytotoxic, anti-inflammatory and anti-oxidant properties (Pican et al., 1986; Gören et al., 2002). Our previous study determined the presence of parthenolide in *n*-hexane extract by TLC (Albayrak et al., 2017), and in the current study, we demonstrated that *n*-hexane extract contains parthenolide by HPLC (Figure 1). Parthenolide is a natural compound belonging to the sesquiterpene lactone family known as germacranolides (Mathema et al., 2012). It is well-known for its potent anti-inflammatory (Kwok et al., 2001) and anti-oxidant properties (Al-Fatlawi et al., 2015). These activities make it a promising candidate for developing new drugs to treat various diseases. Therefore, we suggest that parthenolide contributes to the anti-inflammatory effects of the *n*-hexane extract. Indeed, *n*-hexane extract exhibited similar effects on anti-inflammatory markers as parthenolide. As part of our research, we carried out a toxicogenomic analysis to anticipate how parthenolide interacts with the parameters that we measured by *in vitro* studies. First, genes related to parthenolide and inflammation were identified from the CTD database. Then, common genes were extracted using the MyVenn tool. The initial exploration of CTD data revealed that 197 genes are associated with inflammation, while 66 genes are linked to parthenolide. Nineteen genes were identified as shared between inflammation and parthenolide (Figure 5A).

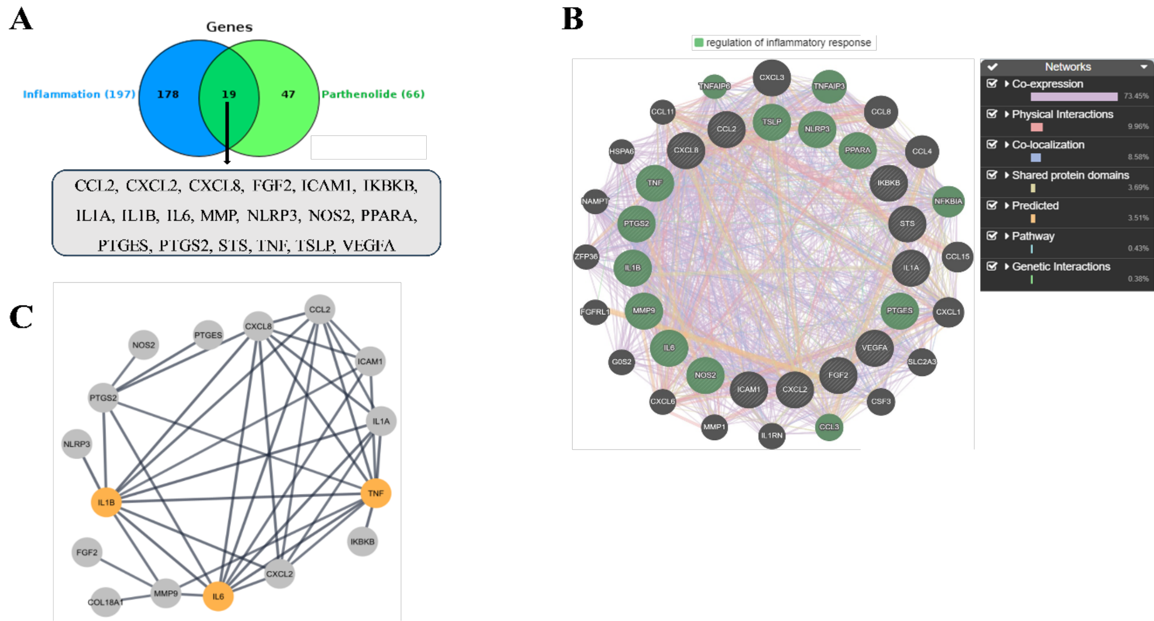

Figure 5. Toxicogenomic analysis. A, Venn diagram shows overlapped genes for inflammation and parthenolide; B, Gene-gene interactions were predicted by GeneMANIA; C, Protein-protein interactions were predicted using the String database and visualized with the Cytoscape software.

The common genes were input into the GeneMANIA online plug-in, generating hypotheses and analyzing the function and interaction of genes, resulting in a connected network. The analysis revealed that co-expressions accounted for 73.45% and were the predominant factors among the overlapping genes. This result suggests that these genes exhibit similar expression levels across conditions in a gene expression study. Nodes in the inner ring represent common genes, while nodes in the outer ring depict genes associated with these common genes, according to GeneMANIA. Additionally, fourteen green nodes indicate genes related to the regulation of the inflammatory response, with a false discovery rate of 5.52e-15 (Figure 5B). Furthermore, the PPIs of the nineteen common genes displayed 19 nodes and 39 edges (Figure 5C), with a PPI enrichment p-value of $<1.0e-16$. In the centrality analysis, the most critical genes among these common genes, related to both parthenolide and inflammation, were identified as IL6, IL-1β, and TNF, ranking in the top three (Table 1).

Table 1. Centrality analysis of common genes between parthenolide and inflammation

Common Genes	Degree	Closeness Centrality	Betweenness Centrality	Number Of Undirected Edges
TNF	10	0.750	0.242	10
IL-1β	10	0.750	0.242	10
IL-6	9	0.714	0.109	9
CXCL8	8	0.625	0.032	8
CCL2	7	0.556	0.004	7
IL1A	6	0.536	0	6
PTGS2	6	0.577	0.257	6
CXCL2	6	0.536	0	6
ICAM1	5	0.517	0	5
MMP9	5	0.556	0.257	5
COL18A1	1	0.366	0	1
FGF2	1	0.366	0	1
NLRP3	1	0.441	0	1
IKBKB	1	0.441	0	1
PTGES	1	0.375	0	1
NOS2	1	0.375	0	1
PPARA	0	0	0	0
STS	0	0	0	0
TSLP	0	0	0	0

We have concluded that the anti-inflammatory properties of *n*-hexane extract may be due to parthenolide, based on both *in silico* and *in vitro* analyses.

CONCLUSION

The findings indicated that exposure to the *n*-hexane extract of *T. argenteum* subsp. *argenteum* reduced the secretion of IL-6, IL-1β, and TNF-α in differentiated THP-1 cells as similar to parthenolide standard. Moreover, toxicogenomic data also demonstrate that parthenolide interacts with these three cytokines, considering the shared genes between parthenolide and inflammation. This study suggests that parthenolide, the significant component of the extract, may be responsible for the anti-inflammatory effects of *n*-hexane extract. Further experiments are being conducted to examine the phytochemical properties and other biological activities of parthenolide and *n*-hexane extract from T. *argenteum* subsp. *argenteum.*

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Concept and Design (EA), resources and materials (EA, FK, GA), data collection and processing (EA, FK, GA, AE), analysis and/or interpretation (EA, FK, AE, GA, IT, EA), literature search (EA, FK, AE, GA, IT, EA), writing (E.A), critical reviews (EA, FK, AE, GA, IT, EA)

REFERENCES

Albayrak G., Nalbantsoy A., Baykan Ş. (2017). In vitro cytotoxic and anti-inflammatory activities of Tanacetum argenteum (lam.) willd. subsp. argenteum extract. Turkish journal of pharmaceutical sciences, 14, 231-236. https://doi.org/10.4274/tjps.42714

Al-Fatlawi, A. A., Al-Fatlawi, A. A., Irshad, M., Rahisuddin, & Ahmad, A. (2015). Effect of parthenolide on growth and apoptosis regulatory genes of human cancer cell lines. Pharmaceutical biology, 53(1), 104–109. https://doi.org/10.3109/13880209.2014.911919

Avula, B., Navarrete, A., Joshi, V. C., & Khan, I. A. (2006). Quantification of parthenolide in Tanacetum species by LC-UV/LC-MS and microscopic comparison of Mexican/US feverfew samples. Die Pharmazie, 61(7), 590–594.

Bhadoriya, S. S., Mishra, V., Raut, S., Ganeshpurkar, A., & Jain, S. K. (2012). Anti-Inflammatory and Antinociceptive Activities of a Hydroethanolic Extract of Tamarindus indica Leaves. Scientia pharmaceutica, 80(3), 685–700. https://doi.org/10.3797/scipharm.1110-09

Bukhari, I. A., Khan, R. A., Gilani, A. U., Shah, A. J., Hussain, J., & Ahmad, V. U. (2007). The analgesic, anti-inflammatory and calcium antagonist potential of Tanacetum artemisioides. Archives of pharmacal research, 30(3), 303–312. https://doi.org/10.1007/BF02977610

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208

Davis, A. P., Wiegers, T. C., Johnson, R. J., Sciaky, D., Wiegers, J., & Mattingly, C. J. (2023). Comparative Toxicogenomics Database (CTD): update 2023. Nucleic acids research, 51(D1), D1257–D1262. https://doi.org/10.1093/nar/gkac833

Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. Immunological reviews, 281(1), 8–27. https://doi.org/10.1111/imr.12621

Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J., Bader, G. D., & Morris, Q. (2018). GeneMANIA update 2018. Nucleic acids research, 46(W1), W60–W64. https://doi.org/10.1093/nar/gky311

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. Nature medicine, 25(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gören, N., Arda, N., & Çaliskan, Z. (2002). Chemical characterization and biological activities of the genus Tanacetum (Compositae). Studies in natural products chemistry, 27, 547-658 https://doi.org/10.1016/S1572-5995(02)80044-8

Jain, N. K., & Kulkarni, S. K. (1999). Antinociceptive and anti-inflammatory effects of Tanacetum parthenium L. extract in mice and rats. Journal of Ethnopharmacology, 68(1-3), 251–259. https://doi.org/10.1016/s0378-8741(99)00115-4

Kany, S., Vollrath, J. T., & Relja, B. (2019). Cytokines in Inflammatory Disease. International journal of molecular sciences, 20(23), 6008. https://doi.org/10.3390/ijms20236008

Kwok, B. H., Koh, B., Ndubuisi, M. I., Elofsson, M., & Crews, C. M. (2001). The anti-inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits IkappaB kinase. Chemistry & biology, 8(8), 759–766. https://doi.org/10.1016/s1074-5521(01)00049-7

Liu, X., Yin, S., Chen, Y., Wu, Y., Zheng, W., Dong, H., Bai, Y., Qin, Y., Li, J., Feng, S., & Zhao, P. (2018). LPS-induced proinflammatory cytokine expression in human airway epithelial cells and macrophages via NF-κB, STAT3 or AP-1 activation. Molecular medicine reports, 17(4), 5484–5491. https://doi.org/10.3892/mmr.2018.8542

Mathema, V. B., Koh, Y. S., Thakuri, B. C., & Sillanpää, M. (2012). Parthenolide, a sesquiterpene lactone, expresses multiple anti-cancer and anti-inflammatory activities. Inflammation, 35(2), 560–565. https://doi.org/10.1007/s10753-011-9346-0

Nisar, A., Jagtap, S., Vyavahare, S., Deshpande, M., Harsulkar, A., Ranjekar, P., & Prakash, O. (2023). Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. Frontiers in pharmacology, 14, 1177050. https://doi.org/10.3389/fphar.2023.1177050

Orhan, I. E., Tosun, F., Gülpınar, A. R., Kartal, M., Duran, A., Mihoglugil, F., Akalgan, D. (2015). LC-MS quantification of parthenolide and cholinesterase inhibitory potential of selected Tanacetum L. (Emend. Briq.) taxa. Phytochemistry letters, 11, 347-352. https://doi.org/10.1016/j.phytol.2014.10.003

Parameswaran, N., & Patial, S. (2010). Tumor necrosis factor-α signaling in macrophages. Critical reviews in eukaryotic gene expression, 20(2), 87–103. https://doi.org/10.1615/critreveukargeneexpr.v20.i2.10

Picman A. (1986). Biological activities of sesquiterpene lactones. Biochemical systematics and ecology, 3(14), 255-281.

Rosselli, S., Bruno, M., Raimondo, F. M., Spadaro, V., Varol, M., Koparal, A. T., & Maggio, A. (2012). Cytotoxic effect of eudesmanolides isolated from flowers of Tanacetum vulgare ssp. siculum. Molecules (Basel, Switzerland), 17(7), 8186–8195. https://doi.org/10.3390/molecules17078186

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research, 13(11), 2498–2504. https://doi.org/10.1101/gr.1239303

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic acids research, 51(D1), D638–D646. https://doi.org/10.1093/nar/gkac1000

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. Cold Spring Harbor perspectives in biology, 6(10), a016295. https://doi.org/10.1101/cshperspect.a016295

Vilhelmova, N., Simeonova, L., Nikolova, N., Pavlova, E., Gospodinova, Z., Antov, G., Galabov, A., & Nikolova, I. (2020). Antiviral, Cytotoxic and Antioxidant Effects of Tanacetum Vulgare L. Crude Extract In Vitro. Folia medica, 62(1), 172–179. https://doi.org/10.3897/folmed.62.e49370

Zhang, Z., Sun, Y., & Chen, X. (2020). NLRC5 alleviated OGD/R-induced PC12-cell injury by inhibiting activation of the TLR4/MyD88/NF-κB pathway. The Journal of international medical research, 48(8), 300060520940455. https://doi.org/10.1177/0300060520940455