Letter to Editor

I Suitable Concentration of Anti-Inflammatory Herbal Extracts in Cell Culture

Moulazadeh A[1*], Kouhpayeh SA[2]

1. Non-Communicable Disease Research Center, Fasa University of Medical Sciences, Fasa, Iran
2. Department of Pharmacology, Fasa University of Medical Sciences, Fasa, Iran

Received: 13 Sep 2020 Accepted: 20 Oct 2020

Dear Editor in chief

Medicinal plants are a rich source of phenolic acids, flavonoids and other secondary metabolites, therefore much research is being done on their anti-inflammatory effects (1). Evaluation of inflammatory responses and immune cells behavior is one of the most important areas of anti-inflammatory activity of herbal extracts. That is usually done in cell culture experiments. Cell culture actually provides an artificial microenvironment to study the immune cells responses and their behavior. The anti-inflammatory effects of herbal extracts in the suppression of signaling pathways and inflammatory mediators (IL-1B, TNF-α, NO, etc.) production is usually investigated in this artificial microenvironment (2, 3). Using appropriate concentration of herbal extract for evaluation of their anti-inflammatory effects is critical. Herbal extracts should not have any inhibitory effect on immune cells viability. It is clear that total production of inflammatory mediators decreases with cell death and damage. Therefore, the reduction of inflammatory mediators cannot be attributed to the anti-inflammatory effects of medicinal plants, definitely (4). In addition, multiple DAMPs[1] are created with cell damage and disrupt the rate and pattern of immune responses in the remaining cells (5).

Recently, an article entitled "Antimicrobial Activity of Scabiosa olivieri Extract and Its Effect on TNF-α and IL-1 Expression in Human Peripheral Blood Cells (PBMCs)" was published in the valued journal of Fasa University of Medical Sciences (6). In the mentioned article, the PBMCs had been treated with two concentrations (4 and 8 mg/ml) of Scabiosa olivieri extract. However, the Scabiosa olivieri extract not only significantly reduced the TNF-α and IL-1 production from PBMCs, but also led to the reduction of the cell viability by 10% and 30% (in MTT assay) respectively in the concentration of 4 and 8 mg/ml. Therefore, the observed anti-inflammatory effects on the production of TNF-α and IL-1 cannot be definitely attributed to the therapeutic effects of the plant. In fact, the herbal concentrations without cytotoxic effect should be used in the evaluation of anti-inflammatory effects. The concentration of herbal medicine within the IC 50 value of cell viability has been used in the field of anti-proliferation effects of cancer and medicinal plants. The purpose of the research is to induce apoptosis and cell cycle arrest (7).

References

1. Lee HN ,Shin SA, Choo GS, Kim HJ, Park YS, Kim BS, et al. Anti‐inflammatory effect of quercetin and galangin in LPS ‐stimulated RAW264. 7 macrophages and DNCB‐induced atopic dermatitis animal models. International journal of molecular medicine. 2018;41(2):888-98.

2. Bai Y, Jiang Y, Liu T, Li F, Zhang J, Luo Y, et al. Xinjiang herbal tea exerts immunomodulatory activity via TLR2/4-mediated MAPK signaling pathways in RAW264.7 cells and prevents cyclophosphamide-induced immunosuppression in mice. Journal of Ethnopharmacology. 2019;228(1):179–87.

***Corresponding Author: Moulazadeh Alireza**, Non-Communicable Disease Research Center, Fasa University of Medical Sciences, Fasa, Iran.
Email: immunomoula@gmail.com
https://Orcid.org/0000-0002-6206-5067

[1] Damage Associated Molecular Patterns

3. Seong-Gu Jeong SG, Sunggyu Kim S, Han Gyung Kim HG, Eunji Kim E, Deok Jeong D, Ji Hye Kim JH, et al. Mycetia cauliflora methanol extract exerts anti-inflammatory activity by directly targeting PDK1 in the NF-κB pathway. Journal of Ethnopharmacology. 2019;231(1): 1–9.
4. Chaoran Song C, Yo Han Hong YH, Jae Gwang Park JG, Han Gyung Kim HG, Deok Jeong D, Junsang Oh J. Suppression of Src and Syk in the NF-κB signaling pathway by Olea europaea methanol extract is leading to its anti-inflammatory effects. Journal of Ethnopharmacology.2019;235(1):38–46.
5. Zindel J, Kubes P. DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. Annual Review of Pathology: Mechanisms of Disease. 2020;15(1):493-518.
6. Alipoor Birgani A, Sartipnia N, Hamdi SMM, Naghizadeh M, Arasteh J. Antimicrobial Activity of Scabiosa Olivieri Extract and Its Effect on TNF-α and IL-1 Expression in Human Peripheral Blood Cells (PBMCs). JFUMS. 2019;9(4):1749-57.
7. Boonyarat C, Jongpremkitpaisan B, Chaiwasukul S, Reubroycharoen P, Waiwut P. The Effects of Thai Traditional Herbal Extracts on the Death of Cancer Cells. Thai Journal of Pharmacology. 2020;42(1):36-47.

Author's Response to the letter to editor

Dear Editor

Inflammation is a very dynamic process, which can be recognized as the first protective response of the immune system. This involves complex interactions of soluble mediators, stationary cells, as well as infiltrating cells and molecules belonging to the extracellular matrix. Successful and controlled inflammatory response is a beneficial process that leads to the clearance of harmful stimuli and the restoration of normal physiology that is precisely regulated by a complex molecular cascade (1). Like all immune responses, it must be controlled, otherwise it can lead to acute diseases such as septic shock and chronic diseases such as atherosclerosis, rheumatoid arthritis, and cancer (2). Note that inhibiting inflammation by removing or inactivating effective inflammatory mediators and cells allows the host to repair damaged tissue (3). Herbal extracts have been used for centuries as a popular remedy for several health disorders. In the field of natural product biology, it has received renewed attention in recent years (4,5). Over the past decade, studies on in vitro and in vivo inflammatory models have led to the identification of natural extracts with proven anti-inflammatory activity (6, 7).

Medicinal plants have anti-inflammatory, anticancer, and antioxidant activities (8). Various studies have shown that plant extracts have different effects depending on which part of the plant is prepared and by which solvent (9, 10). For example, Kotakadi et al. have shown that leaf extract of *Ginkgo biloba EGb 761* can suppress the activation of macrophages and drives apoptosis of the CD4+ effector T cell population (11). Earlier studies have also proven the apoptotic effects of Arctium lappa L.dichloromethanic and hydroethanolic root extracts [12,13] Gurunanselage et al. also showed that apart from the anti-inflammatory effect, the extract of Arctium lappa L. can cause apoptosis in Jurkat human leukemic T cells (14). Another study showed that Terminalia Arjuna prevents the formation of foam cells and increases the apoptosis of foam cells and macrophage (15). Therefore, different results show that the effect of plant extracts on cells in the same concentrations can be different, and as mentioned before, the most important way to control inflammation and repair damaged tissues is to remove or inactivate effective inflammatory mediators and cells (3).

In our study, we used all plant components (roots, flowers, stems and leaves). The main purpose of this study was to obtain the concentration of the extract to investigate the effect on healthy and cancer cells (data not shown).

Therefore, the results of this study showed that even if IC50 concentration is used, the viability percentage of PBMCs decreases. Since PBMCs are a collection of lymphocytes and monocytes, determining which cell is most affected requires further research, such as flow cytometric analysis.

Therefore, the decrease in the level of inflammatory cytokines such as TNF-α and IL-1 may be due to decrease in cell number or decrease in cell activity, which in any case will reduce the inflammatory process.

References

1. Medzhitov R.Inflammation 2010: new adventures of an old flame. Cell. 2010; 140(6):771-6. doi: 10.1016/j.cell.2010.03.006.
2. Vinuesa CG, Preiss T. Inflammation: gone with translation. PLoS Genet. 2014;10(6):e1004442. doi: 10.1371/journal.pgen.1004442. eCollection 2014 Jun.
3. Fujiwara N, Kobayashi K. Macrophages in inflammation. Curr Drug Targets Inflamm Allergy. 2005;4(3):281-6. doi: 10.2174/1568010054022024.
4. Bremner P, Heinrich M. Natural products as targeted modulators of the nuclear factor-kappaB pathway.
J Pharm Pharmacol. 2002;54(4):453-72. doi: 10.1211/0022357021778637.
5. Geetha S, Sai Ram M, Singh V, Ilavazhagan G, Sawhney RC. Anti-oxidant and immunomodulatory properties of seabuckthorn (Hippophae rhamnoides)--an in vitro study. J Ethnopharmacol. 2002;79(3):373-8. doi: 10.1016/s0378-8741(01)00406-8.
6. Sultana N, Saify ZS. Naturally occurring and synthetic agents as potential anti-inflammatory and immunomodulants. Antiinflamm Antiallergy Agents Med Chem. 2012;11(1):3-19. doi: 10.2174/187152312803476264.
7. Orlikova B, Legrand N, Panning J, Dicato M, Diederich M. Anti-inflammatory and anticancer drugs from nature. Cancer Treat Res. 2014;159:123-43. doi: 10.1007/978-3-642-38007-5_8.
8. Siriwatanametanon N, Fiebich BL, Efferth T, Prieto JM, Heinrich M. Traditionally used Thai medicinal plants: in vitro anti-inflammatory, anticancer and antioxidant activities. J Ethnopharmacol. 2010;130(2):196-207. doi: 10.1016/j.jep.2010.04.036. Epub 2010 May 8.
9. Maione F, Russo R, Khan H, Mascolo N. Medicinal plants with anti-inflammatory activities. Nat Prod Res. 2016;30(12):1343-52. doi: 10.1080/14786419.2015.1062761. Epub 2015 Jul 29.
10. Prieto JM, Recio MC, Giner RM, Máñez S, Giner-Larza EM, Ríos JL. Influence of traditional Chinese anti-inflammatory medicinal plants on leukocyte and platelet functions. J Pharm Pharmacol. 2003;55(9):1275-82. doi: 10.1211/0022357021620.
11. Kotakadi VS, Jin Y, Hofseth AB, Ying L. Ginkgo biloba extract EGb 761 has anti-inflammatory properties and ameliorates colitis in mice by driving effector T cell apoptosis. Carcinogenesis. 2008;29(9):1799-806. doi: 10.1093/carcin/bgn143. Epub 2008 Jun 20.
12. Ghafari F, Rajabi MR, Mazoochi T, Taghizadeh M, Nikzad H, Atlasi MA, Taherian A. Comparing Apoptosis and Necrosis Effects of Arctium Lappa Root Extract and Doxorubicin on MCF7 and MDA-MB-231 Cell Lines. Asian Pac J Cancer Prev. 2017;18(3):795-802. doi: 10.22034/APJCP.2017.18.3.795.
13. Predes FS, Ruiz AL, Carvalho JE, Foglio MA, Dolder H. Antioxidative and in vitro antiproliferative activity of Arctium lappa root extracts. BMC Complement Altern Med. 2011;11:25. doi: 10.1186/1472-6882-11-25.
14. Gurunanselage Don RAS, Yap MKK. Arctium lappa L. root extract induces cell death via mitochondrial-mediated caspase-dependent apoptosis in Jurkat human leukemic T cells. Biomed Pharmacother. 2019;110:918-929. doi: 10.1016/j.biopha.2018.12.023. Epub 2018 Dec 17.
15. Bhansali S, Khatri S, Dhawan V. Terminalia Arjuna bark extract impedes foam cell formation and promotes apoptosis in ox-LDL-stimulated macrophages by enhancing UPR-CHOP pathway. Lipids Health Dis . 2019;18(1):195. doi: 10.1186/s12944-019-1119-z.

نامه به سردبیر

غلظت مناسب عصاره گیاهان دارویی در مهار پاسخ های التهابی در فاز کشت سلولی

علیرضا مولازاده[1*]، سید امین کوهپایه[2]

۱- مرکز تحقیقات بیماری‌های غیرواگیر، دانشگاه علوم پزشکی فسا، فسا، ایران
۲- بخش فارماکولوژی، دانشگاه علوم پزشکی فسا، فسا، ایران

تاریخ دریافت مقاله: ۱۳۹۹/۰۶/۲۳ تاریخ پذیرش مقاله: ۱۳۹۹/۰۷/۲۹

***نویسنده مسئول: علیرضا مولازاده**، مرکز تحقیقات بیماری‌های غیرواگیر، دانشگاه علوم پزشکی فسا، فسا، ایران.
Email: immunomoula@gmail.com
https://Orcid.org/0000-0002-6206-5067

مجله دانشگاه علوم پزشکی فسا | سال دهم | شماره ۳| پاییز ۱۳۹۹|صفحه: ۲۳۹۶-۲۳۹۹