ABSTRACT
Background: Herbomineral formulations are momentous in an audience of worldwide by virtue of their holistic approach to life. These formulations are widely used as complementary therapies in immunocompromised patients including cancer. Still, there is the need of cost-effective and safe herbomineral-based formulation that can modulate immune response by the regulation of cytokines cascades. Objective: Current study, we investigated immunomodulatory effect of TEBEH in LPS-induced cytokines expression levels in mouse splenocytes in vitro. Materials and Methods: The most effective and safe concentrations of TEBEH were chosen by determining the cell viability of splenocytes using MTT assay. The pro-inflammatory cytokines such as TNF-α, IL-1β, MIP-1α, and IFN-γ were measured in cell supernatants using ELISA. Results: MTT data showed TEBEH formulation was found safe up to 10.53 μg/mL. At noncytotoxic concentrations (0.0001052–10.53 μg/mL), TEBEH significantly (P < 0.001) inhibited the expressions of TNF-α, IL-1β, and MIP-1α in mouse splenocytes as compared with vehicle control. Conclusion: In summary, TEBEH may indeed promote an anti-inflammatory environment by suppression of pro-inflammatory cytokines. These observations indicated that TEBEH has potential effects in downregulating the immune system and might be developed as a useful anti-inflammatory product for various inflammatory disorders.

Key words: Inflammation, immunomodulation, splenocytes, pro-inflammatory cytokines, ELISA

SUMMARY
The present study was undertaken to evaluate an immunomodulatory effect of the herbomineral formulation in LPS-induced mouse splenocytes with the measurement of cytokines expression such as TNF-α, IL-1β, MIP-1α and IFN-γ. The results showed that the expression of TNF-α, IL-1β, and MIP-1α was significantly down-regulated while, IFN-γ was significantly up-regulated in mouse splenocytes. It is hypothesized that modulation of the proinflammatory cytokines might occur via NFκB pathway. Therefore, the herbomineral test formulation might act as an effective anti-inflammatory and immunomodulatory product, and this can be used as a complementary and alternative treatment for the prevention of various types of inflammatory and auto-immune disorders.

INTRODUCTION
Cytokines are the key factors in acute and chronic inflammation. Inflammation is characterized by an interplay between pro- and anti-inflammatory cytokines. The anti-inflammatory cytokines are a series of immunoregulatory molecules that control the pro-inflammatory cytokine response.[12] The narrow therapeutic range and serious adverse effects of immunosuppressive drugs have proved almost insurmountable obstacles.[13] As a consequence, the finding of lead compound with markedly lower toxicity and higher immunosuppressive activity is of great interest. In recent years, medicinal plants that have been practiced for thousands of years in clinic provide a vast source of pharmaceutical material for the development of effective drugs and offer some unique advantages with low toxicity profiles.[14] Many herbal extracts either per se or in combinations with medicinal plants or with minerals may have activities on cytokines.[15] Herbal medicinal preparations can favorably regulate the whole immune system.[17] Therefore, anti-inflammatory phytomedicines may be beneficial for the management of chronic inflammatory disorders due to overactivated immunity.[19] Vast scientific studies are going on toward dietary phytochemicals that have played a significant role in drug discovery and development, especially in the case of antiproliferation, cytotoxic, and immunomodulatory effects.[19] The plant-based immunomodulators are gaining special interest, since their possible use in modern medicine was suggested.[10] TEBEH is a novel proprietary herbomineral formulation consisting of four
ingredients; an herbal extract (ashwagandha root extract) along with a mixture of minerals (zinc chloride, magnesium gluconate hydrate, and sodium selenate). Several reports have demonstrated its potent antibacterial, immunomodulatory, and antitumor activity due to the presence of withanolides as major active ingredient.\textsuperscript{[11,12]} Zinc potentiates the immunomodulatory effect by increasing the levels of leukocyte count and the phagocytic index.\textsuperscript{[13]} Sodium selenate had exhibited the level of the phagocytic index and restored the level of IL-1, IL-6, and TNF-α near to normal.\textsuperscript{[14]} The selenium-containing compounds are biologically active and can modify neutrophil functions.\textsuperscript{[15]} Magnesium reduces the production of an inflammatory cytokine through activation of NF-κB pathways, which is a novel innate immunomodulatory mechanism.\textsuperscript{[16]} The different type of immune cells such as dendritic cells, macrophages, and the spleen can play an important role to stop the acute/chronic inflammation and retrieve a steady-state strategy through the secretion of immunomodulating cytokines.\textsuperscript{[17,18]} These immune cells have been reported to be useful as cellular models for in vitro studies. Therefore, authors used splenocytes to assess the effect of the herbomineral formulation TEBEH on in vitro cell culture. In market, several herbal extracts and synthetic immunomodulatory agents are available for the management of various patients suffering from autoimmune diseases, allergies, organ transplantations, and systemic immunocompromise. However, so far, there is no report published about the immunomodulatory activity of Withania somnifera extract (synonym Indian ginseng) based herbomineral formulation, TEBEH. Therefore, the aim of this study was to illustrate the immunomodulatory activity of TEBEH in lipopolysaccharide (LPS)-induced mouse splenocytes with the estimation of defined pro-inflammatory cytokines expression level.

MATERIALS AND METHODS

Chemicals

MTT, LPS, 1-glutamine, RPMI-1640, penicillin, HEPES, streptomycin, 2-mercaptoethanol, and rapamycin were purchased from Sigma Chemical Co., St. Louis, MO. ELISA kits for all cytokines such as TNF-α, MIP-1α, and IL-1β were purchased from R&D Systems, USA. FBS were procured from GIBCO, USA. W. somnifera (commonly known as ashwagandha) root extract powder was procured from Sanat Products Ltd., India. The root extract was identified and authenticated by Dabur Research Foundation (DRF), Ghaziabad, India, with voucher number (DOM_EXC/E63). Zinc chloride and magnesium(II) gluconate hydrate were procured from TCI, Japan. Sodium selenate was procured from Alfa Aesar, USA.

Experimental design

The experiment was designed into seven groups. Group 1 contained the splenocyte cells without LPS, denoted as normal control (NC). Group 2 was served as stimulant group that included cells, DMSO along with LPS defined as vehicle control (VC), i.e., negative control. Group 3 was defined as positive control, rapamycin (1 nM). The test item group that included splenocyte cells with LPS along with TEBEH at various concentrations of 0.00001053–10.53 μg/mL.

Composition and preparation of herbomineral product, TEBEH

The required quantity of each individual ingredient was mixed together with the concentration of 37.64 mg/mL TEBEH. The composition of each ingredients selected is as follows: zinc chloride (1.04 mg/mL), sodium selenate (1.195 μg/mL), magnesium gluconate (170.6 mg/mL), and ashwagandha root extract powder (≥ 5% of total withanolides, 200 mg/mL). The above formulation was vortexed to achieve a homogenous solution, which was considered as 100% stock solution. The above stock solution was further diluted in serum-free medium (SFM) to obtain a range of concentrations (in % v/v) for subsequent treatment.

Animal care and housing

C57BL/6 male mouse (8 weeks old) was purchased from Ms. Vivo Biotech Ltd., Hyderabad, India. Rodent laboratory chow diet and drinking tap water were provided ad libitum and maintained under controlled conditions a temperature of 22 ± 3°C, humidity of 30–70% and a 12-h light/12-h dark cycle. The approval of the institutional animal ethics committee (IAEC) was obtained before carrying out the animal experiment.

Mouse splenocyte cultures

C57BL/6 male mouse was sacrificed, and the spleen was removed and ground by passing through a sterile plastic strainer under aseptic conditions. After that, the cells were centrifuged twice at 1000g for 5 min. Cells were lysed by lysis buffer (0.15 M NaCl, 0.01 M NaHCO\textsubscript{3}, and 0.1 mM EDTA, pH 7.4) and then the cell pellets were washed twice with RPMI-1640 medium. Further, the cells were resuspended in a sterile RPMI-1640 medium (RPMI-1640 medium plus 10% FBS, 2 mM glutamine, streptomycin and 100 IU/mL penicillin, 15 mM HEPES, and 50 mM 2-mercaptoethanol). The cell counts were performed with the help of hemocytometer and cell viability was determined using trypan-blue dye exclusion technique. The cells were cultured in 96-well tissue culture plates with 0.2 × 10\textsuperscript{6} cells well per well. They were incubated at 37°C in a humidified atmosphere containing 5% CO\textsubscript{2} for the specified period.

Cell culture and test item (TEBEH) treatment

The splenocyte (0.2 × 10\textsuperscript{6} cells per well) were grown in 96-well culture plates using RPMI-1640 medium supplemented with 10% FBS, 100 μg/mL of streptomycin, and 100 IU/mL of penicillin. The LPS (0.5 μg/mL) induced splenocyte cells cultures were grown for 48 h at 37°C in a humidified CO\textsubscript{2} incubator (5% CO\textsubscript{2}). The effect of cytotoxicity was determined by exposing cells to different concentrations of TEBEH in a RPMI-1640 medium. The various concentrations of TEBEH were used from 0.00001053–10.53 μg/mL in splenocytes cell culture. The respective VC kept in the assay was DMSO with LPS.

MTT assay

Cytotoxicity was determined by exposing cells to different concentrations of TEBEH in a RPMI. The respective VC kept in the assay was DMSO with LPS. The number of viable cells was estimated based on the conversion of MTT to formazan dye using a mitochondrial enzyme. The effect of TEBEH on cell viability of splenocytes was determined with the help of the following equation:

\[
\text{Cell viability} = \frac{\text{OD of control cells} - \text{OD of cells treated with TEBEH}}{\text{OD of control cells}} \times 100
\]

where % Cytotoxicity = [(O.D. of control cells – O.D. of cells treated with TEBEH)/OD of control cells] × 100

Cytokines assay

The effect of herbomineral-based formulation TEBEH on the production of TNF-α, IL-1β, MIP-1α, and IFN-γ were measured by ELISA method using culture supernatants using a Biotech reader (SIAFRT/Synergy HT multimode reader). For the estimation of TNF-α, IL-1β, MIP-1α, and IFN-γ in LPS (0.5 μg/mL) induced splenocyte were exposed to TEBEH at selected nontoxic concentrations (0.00001053–10.53 μg/mL). After 48h of incubation, supernatants were analyzed for the secreted levels of cytokines using ELISA as per manufacturer’s instructions.\textsuperscript{[17-19]}
STATISTICAL ANALYSIS

Data analysis was performed with Sigma Plot Statistical Software (Version 11.0). Differences between means (in triplicates) were assessed for statistical differences using one-way analysis of variance (ANOVA) and Student’s t-test. *P* less than 0.05 was statistically significant. The results are shown as mean ± standard error of mean (SEM).

RESULTS

Assessment of *in vitro* immune cells viability by MTT assay

The concentration that resulted in more than 150% viability was selected for subsequent cytokines estimation. The normal splenocyte cells and VC groups showed 100% cell viability. The rapamycin showed 136.52% cell viability at 1 nM. The percentage cell viability was increased in all the tested concentrations (0.00001053–10.05 µg/mL) with respect to the VC group, which might be due to proliferation in cell culture [Figure 1].

Expression of TNF-α in mouse splenocytes

The results of TEBEH demonstrated a significant suppression of TNF-α levels by 12.80, 13.69, 22.22% (*P* ≤ 0.001), 24.31% (*P* ≤ 0.001), and 30.46% (*P* ≤ 0.001) at the tested concentrations, i.e., at 0.0001053, 0.01053, 0.1053, 1.053, and 10.53 µg/mL, respectively, as compared with the VC. The test item at high concentration (10.53 µg/mL) with respect to rest of the tested concentrations showed better response by suppressing the level of TNF-α [Figure 2].

Expression of IL-1β in mouse splenocytes

The level of IL-1β in the normal control (NC) group was 19.76 ± 1.13 pg/mL and significantly increased by 79.10% in the VC group (35.37 ± 3.94 pg/mL) after induction with LPS. The test item TEBEH showed a significant (*P* ≤ 0.05) inhibition of IL-1β secretion at the two highest tested concentrations, i.e., at 0.01053 and 0.1053 µg/mL by 34.24 and 37.23%, respectively, as compared with the VC group [Figure 3].

Modulation of MIP-1α expression in mouse splenocytes

The test item TEBEH showed significant (*P* ≤ 0.001) inhibition of MIP-1α secretion by 24.27, 41.30, 45.72, and 18.11% at the tested concentrations, i.e., at 0.0001053, 0.001053, 0.01053, and 0.01053 µg/mL, respectively, as compared with the VC [Figure 4].

[Figure 1: Measurement of cytotoxicity by MTT assay in splenocyte cells. Values are represented as mean ± SEM (triplicates). NC: Normal control; RAP: Rapamycin (1 nM); VC: Vehicle control]

[Figure 2: Effect of TEBEH on TNF-α secretion in LPS-mediated splenocyte cells was measured 48 h after exposure. ***P* ≤ 0.001 vs. VC and **P* ≤ 0.01 vs. NC (using one-way ANOVA)]

[Figure 3: Inhibition of LPS-mediated production of IL-1β by TEBEH. *P* ≤ 0.01 vs. VC (using one-way ANOVA)]

[Figure 4: Effect of TEBEH on the expression of MIP-1α in LPS-mediated splenocytes. ###*P* ≤ 0.001 vs. NC and ***P* ≤ 0.001 vs. VC (using one-way ANOVA)]

[Figure 5: Dose-dependent inhibition of LPS-mediated production of IFN-γ by TEBEH. *P* = 0.01 vs. VC and **P* ≤ 0.05 vs. NC (using paired t-test)]
Expression of IFN-γ in mouse splenocytes

The test product TEBEH demonstrated an elevation of IFN-γ as compared with the LPS-stimulated VC group. The result showed the expression of IFN-γ was significantly increased by 21.58, 25.79, and 32.22% (P ≤ 0.01) at 0.1053, 1.053, and 10.53 µg/mL respectively, as compared to the VC [Figure 5].

DISCUSSION

Cytokines play a key role in immunomodulation. A hallmark of immunity is the production of a multifaceted array of inflammatory cytokines. Disease progression or regression could be possible by estimation the up- and/or downregulation of cytokine signaling cascades in response to pathologic or therapeutic interventions.[20-21] Immunomodulators can modify the activity of immune function through the impulsive modulation of cytokines.[22,23] Infection and tissue injury results in alterations in host metabolic and immune homoeostasis. These changes result in the secretion of endogenous mediators from a complex cascade of mononuclear phagocyte process. Among these, the most vital host proteins are called cytokines.[24]

The use of herbomineral products to maintain or improve health has gradually increased across the globe over the last couple of years. Moreover, formulating new products that have the ability to improve the overall health by reducing inflammation is essential because of the potential for long-term effectiveness, decreased toxicities, and lower costs. Therefore, mouse splenocytes was selected as test system to study immune responses and to investigate the anti-inflammatory effects of a newly developed proprietary herbomineral product TEBEH. The TEBEH is a novel proprietary herbomineral formulation containing a mixture of herbal extract like ashwagandha and three minerals, viz., zinc chloride, magnesium gluconate hydrate, and sodium selenate.

The rational for selection of each constituents in the TEBEH based on the immunomodulatory activity through same central signaling pathways of the selected component per se with specific salt to get the desired solubility of the formulation. The possible anti-inflammatory mechanisms of TEBEH are shown in Figure 6. The metabolic activity is evaluated by measuring the activity of a mitochondrial enzyme succinate dehydrogenase using the MTT test. This test is widely used in the in vitro evaluation of the toxicity of any test item.[25] On the basis of the cell viability using MTT assay, we showed that TEBEH was found to be safe at all the tested concentrations with increased percentage viability ranges from 154.57 to 187.17%. Hence, all the tested concentrations were selected for the estimation of cytokines. The maximum cell viability was reported as 187.17% at 0.1053 µg/mL [Figure 1]. It can be concluded that the test item showed an increased cell viability at the specified concentrations with respect to the both normal and VC groups. The pro-inflammatory cytokines TNF-α play a central role in inflammation,[26] immune modulation,[27] and lymphocyte activation.[28] In most of the immune-mediated disorders, TNF-α was anticipated as the major factor that controls many disease pathologies.[29] The role of TNF-α and its alteration was significantly reported to improve insulin resistance, lipid profiles, and so on, in chronic inflammatory diseases patients.[30] Our results showed a significant downregulation of the expression of TNF-α at the concentration ranging from 0.1053 to 10.53 µL. It is evident that the herbomineral formulation TEBEH could be used against inflammatory disorders by regulating the expression of TNF-α. The importance of IL-1β expression in immunologic and inflammatory functions during infections is well established.[31] Results exhibited a significant inhibition of the expression of IL-1β at the concentrations ranging from 0.01053 to 10.53 µL. The inhibitory effect of TEBEH might play an important role in mediating auto-inflammatory diseases as a complementary and alternate medicine. The results suggest that at higher concentration TEBEH showed better immunosuppressive activity with respect to lower tested concentrations. MIP-1α plays an important role in mediating the acute inflammatory response in trauma hemorrhage and reported that MIP-1α reduction could be beneficial in minimizing the inflammatory responses in several diseases.[32] The data revealed that MIP-1α level was significantly suppressed at all the tested concentrations after exposure of the novel herbomineral formulation TEBEH in splenocyte cells.

Numerous literatures suggest that IFN-γ expression play a key role in the regulation of visceral adipose tissue inflammatory response,[33] regulate glucose homeostasis,[34] and inhibit the inflammatory response of macrophage cells[35] and many other important inflammatory disorders. This study showed that the level of IFN-γ was significantly increased at higher concentration (10.53 µg/mL). Blanchard et al. reported that bacterial LPS had induced the expression of pro-inflammatory cytokine IFN-α/β in fresh splenocytes culture by stimulation of the B lymphocytes and macrophages. Because the induction of IFN-γ by bacterial LPS may play an important role in resistance/recovery mechanisms against bacterial infections,[36] it also emphasized that IFN-γ has the bactericidal and cytotoxic potential of macrophages. From this experiment in the VC group, the level of IFN-γ expression was remarkably increased; which might be due to influence of bacterial LPS. Besides, increased plasma level of IFN-γ has been evident in acute HIV infection that determines the inflammatory cytokine responses.[37] Increasing levels of IFN-γ can be defined as crucial macrophage activator and also upregulate macrophage antimicrobial activity.[38] Thus, IFN-γ possibly is upregulated due to upregulation of STAT4 expression through Th1 CD4 T-cell differentiation.[39] Therefore, it can be concluded that the tested herbomineral formulation TEBEH would be able to increase the production of IFN-γ, which might be used as a supplement in the inflammatory disorders. For instance, ashwagandha was shown to inhibit TNF-α-induced nuclear factor-kappa B (NF-κB) activation in human myelomonoblastic leukemia cells.[40,41] zinc has the ability to induce or inhibit the activation of NF-κB[42] and magnesium plays a critical regulatory role in NF-κB activation.[43] Overall, the herbomineral-based formulation, TEBEH, remarkably downregulates the expression of the pro-inflammatory cytokines like TNF-α, MIP-1α, and IL-1β and upregulates IFN-γ in splenocyte cells.

CONCLUSION

The study results summarize that TEBEH showed better and significant inhibition of pro-inflammatory cytokines (TNF-α and IL-1β) and
chemokine (MIP-1α) expression as compared with the VC group in mouse splenocyte cells. In brief, the expression of MIP-1α was suppressed at all the tested concentrations. Additionally, the level of IFN-γ was significantly upregulated in the highest concentration. Overall, data indicated a significant reduction of TNF-α, MIP-1α, and IL-1β and elevation of IFN-γ the pro-inflammatory mediators upon exposure with the TEBEH on splenocyte cells. In conclusion, the herbomineral-based formulation TEBEH might act as an effective anti-inflammatory product and can be used as a complementary and alternative treatment and prevention of various types of inflammatory disorders.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Opal SM, De Palo VA. Anti-inflammatory cytokines. Chest 2000;117:1162-72.
2. Cavallion JM. Pro-versus anti-inflammatory cytokines: myth or reality. Cell Mol Biol (Nice-les-grand) 2001;47:695-702.
3. Dinarello CA. Proinflammatory cytokines. Chest 2000;118:503-8.
4. Wong SH. Therapeutic drug monitoring for immunosuppressants. Clin Chim Acta 2001;312:241-53.
5. Serkova N, Brand A, Christians U, Leibfritz D. Evaluation of the effects of immunosuppressants on neuronal and glial cells in vitro by multinuclear magnetic resonance spectroscopy. BBA-Mol Cell Res 1998;1314:93-104.
6. Burns JJ, Zhao L, Taylor EW, Spelman K. The influence of traditional herbal formulas on cytokine activity. Toxicology 2010;278:140-59.
7. Speelman K, Burns J, Nichols D, Winters N, Ottersberg S, Tenborg M. Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. Alternat Med Rev 2006;11:128-50.
8. Haddad PS, Azar GA, Groom S, Boivin M. Natural health products, modulation of immune function and prevention of chronic diseases. Evid Based Complement Alternat Med 2005;2:513-20.
9. Yeo SK, Omar AR, Ho WW, Boon Beh BK, Ali AM, Altheen NB. Immunomodulatory effect of Raphithodora korthalaisi on mice splenocyte, thymocyte and bone marrow cell proliferation and cytokine expression. Afr J Biotechnol 2006;10:10744-51.
10. Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, Dutt P. Studies on the antioxidant and immunomodulatory mechanism. J Immunol 2012;188:6338-46.
11. Mosmann TR, Sad S. The expanding universe of T cell subsets: Th1, Th2 and more. Immunol Today 1996;17:138-46.
12. Chen K, Jay K. T cell-mediated host immune defenses in the lung. Annu Rev Immunol 2013;31:605-32.
13. Durieux M, Maury-Brachet R, Girardin M, Rochard E, Estuaries AB. Contamination by heavy metals (Cd, Zn, Cu, and Hg) of eight fish species in the Gironde estuary (France). Estuaries 2015;28:819-1.
14. Whiteside TL. Introduction to cytokines as targets for immunomodulation. In: Cytokines in human health part of the series methods in pharmacology and toxicology. Humana Press Inc. Totowa, NJ 2007:1-15.
15. Wahab S, Hussain A. Cytokines as targets for immunomodulation. Int J Pharm Sci 2013;5:60-4.
16. Hussain A, Shadmehr M, Maksood A, Ansari SH. Protective effects of Picrorhiza kurroa on cyclophosphamide-induced immunosuppression in mice. Phcog Res 2013;5:305-6.
17. Ilays U, Katate DP, Aeri V, Nasser PP. A review on hepatoprotective and immunomodulatory herbal plants. Phcog Rev 2016;10:66-70.
18. Molloy RG, Mannick JA, Rodrick ML. Cytokines, sepsis and immunomodulation. Br J Surg 1993;80:289-97.
19. Zhang JM, An J. Cytokines, inflammation and pain. Int Anesthesiol Clin 2007;45:27-32.
20. Sibi G, Rabina S. Inhibition of pro-inflammatory mediators and cytokines by Chlorella vulgaris extracts. Phcog Res 2016;8:119-22.
21. Kmal A, Lououzi B, Zaid H, Imtira H, Saad B. In vitro evaluation of anti-inflammatory and antioxidant effects of Asparagus aphyllus L., Categacus auriculatus L., and Ephedra alata-Decne, in mononucleocytes and cultures of HepG2 and THP-1-derived macrophages. Pharmacog Commun 2017;2:74-33.
22. Bradley JR. TNF-mediated inflammatory disease. J Pathol 2008;214:149-60.
23. Popa C, Natea MG, van Riel PL, van der Meer JW, Stalenhout A. The role of TNF-alpha in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. J Lipid Res 2007;48:751-62.
24. Gao B, Radaeva S, Park O. Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. J Leukoc Biol 2009;86:513-28.
25. Singh D, Aggarwal A, Maurya R, Naik S. Withania somnifera inhibits NF-κB and AP-1 transcription factors in human peripheral blood and synovial fluid mononuclear cells. Phytother Res 2007;21:905-13.
26. Hsieh CH, Fink M, Hsieh YC, Kan WH, Hsu JT, Schwacha MG, et al. The role of MIP-1α in the development of systemic inflammatory response and organ injury following trauma hemorrhage. J Immunol 2006;181:2806-12.
27. Zhang H, Potter BJ, Cao JM, Zhang C. Interferon-gamma induced adipose tissue inflammation is linked to endothelial dysfunction in type 2 diabetic mice. Basic Res Cardiol 2011;106:1135-45.
28. O’Rourke RW, White AE, Metcalf MD, Winters BR, Diggs BS, Zhu X, et al. Systemic inflammation and insulin sensitivity in obese IFN-γ knockout mice. Metabolism 2012;61:1152-61.
29. Luu XY, Takahara T, Kawai K, Fujino M, Sugiyama T, Tsuneyama K, et al. IFN-γ deficiency attenuates hepatic inflammation and fibrosis in a steatohepatitis model induced by a methionine- and choline-deficient high-fat diet. Am J Physiol Gastrointest Liver Physiol 2013;305:G991-9.
30. Blanchard DK, Djeu JY, Klein TW, Friedman H, Stewart WE. 2nd. Interferon-gamma induction by lipopolysaccharide: dependence on interleukin 2 and macrophages. J Immunol 1988;136:963-70.
31. Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, et al. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. J Virol. 2009;83:3719-33.
32. Zhou J, Nagarapittha Z, Zheng Y, Nagaragana M. Immune modulation by chondroitin sulphate and its degraded disaccharide product in the development of an experimental model of multiple sclerosis. J Neuroimmunol 2010;223:55-64.
33. Sano C, Sato K, Shinizu T, Kajitani H, Kawauchi H, Tomioka H. The modulating effects of proinflammatory cytokines interferon-gamma (IFN-g) and tumour necrosis factor alpha (TNFα), and immunoregulating cytokines IL-10 and transforming growth factor beta (TGFβ), on anti-microbial activity of murine peritoneal macrophages against Mycobacterium avium intracellulare complex. Clin Exp Immunol 1999;115:435-42.
34. Lawrence T. The nuclear factor kB (NF-kB) pathway in inflammation. Cold Spring Harb Perspect Biol 2009;1:a001651.
35. Singh S, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. J Biol Chem 1995;270:24995-5000.
36. Kruse-Jarres JD. The significance of zinc for humoral and cellular immunity. J Trace Elem Electrolytes Health Dis 1989;3:1-8.