RESEARCH ARTICLE / *Araştırma Makalesi*

Investigation of the Effects of Hydroxyzine Hydrochloride on Mammalian Macrophages

Hidroksizin Hidroklorürün Memeli Makrofajları Üzerindeki Etkilerinin İncelenmesi

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Abstract

Objective	Hydroxyzine hydrochloride is an antihistamine-derived drug that has been widely preferred in the treatment of anxiety disorder, mental distress and stress in recent years. There are not enough studies in the literature on the effects of hydroxyzine hydrochloride on the immune system.
Materials and Methods	In our study, we investigated the effects on proinflammatory cytokine levels by administering 1, 5, 10 and 20 µg/mL hydroxyzine hydrochloride in the presence and absence of lipopolysaccharides (LPS) danger signal in J774.2 macrophage cell line. Student's t test was used for statistical analysis ($p<0.0001$, N=3) All experimental conditions were tested in triplicate.
Results	Hydroxyzine hydrochloride did not affect the cytokine levels of tumor necrosis factor α (TNFα), interleukin-6 (IL-6), interleukin 12p40 (IL12p40) and granulocyte-macrophage colony-stimulating factor (GM-CSF) after 24 hours of incubation in macrophage cells and did not activate immune cells. There was no statistical difference in this values, which did not show an immunostimulatory effect.
Conclusion	Since hydroxyzine hydrochloride does not have an immunostimulatory effect for macrophage cells, it can be used safely in the clinic.
Keywords	Hydroxyzine Hydrochloride; Macrophage;J774.2 Cell Line; Cytokine

Öz

Amaç	*Hidroksizin hidroklorür son yıllarda anksiyete bozukluğu, ruhsal sıkıntı ve gerginliğin tedavisinde yaygın olarak tercih edilen antihistaminik türevli bir ilaçtır. Literatürde Hidroksizin hidroklorürün bağışıklık sistemi üzerine etkileri ile ilgili yeterli çalışma mevcut değildir*
Gereç ve Yöntemler	*Çalışmamızda J774.2 makrofaj hücre hattında lipopolisakkarit (LPS) tehlike sinyali varlığında ve yokluğunda 1, 5,10 ve 20 µg/ml hidroksizin hidroklorür uygulayarak proinflamatuvar sitokin seviyelerine etkilerini araştırdık. İstatistiksel analiz için Student t testi uygulandı ($p<0.0001$, N=3.) Tüm deney koşulları üçlü olarak test edildi.*
Bulgular	*Hidroksizin hidroklorür makrofaj hücrelerinde 24 saatlik inkübasyondan sonra tümör nekroz alfa (TNFα), interlökin 6 (IL-6), interlökin-12 subunit beta (IL12p40) ve granülosit makrofaj koloni uyarıcı faktör (GM-CSF) sitokin seviyelerini etkilemeyerek bağışıklık hücrelerini aktive etmedi. İmminostimülatör etki göstermeyen bu değerlerde istatistiksel olarak bir fark gözlenmedi.*
Sonuç	*Hidroksizin hidroklorür makrofaj hücreleri için imminostimülatör etki göstermediği için klinikte güvenli bir şekilde kullanılabilinir.*
Anahtar Kelimeler	*Hidroksizin Hidroklorür, Makrofaj, J774.2 Hücre Hattı, Sitokin*

INTRODUCTION

In recent years, there has been an intense use of antidepressant drugs around the world. There are numerous clinical studies showing that when these drugs are used under the supervision of a psychiatrist in psychological disorders and diseases, they experience regression in symptoms. Due to the high number of patients coming to mental health professionals and to achieve rapid results, they prefer psychopharmacotherapy instead of psychotherapy. Today, people apply to antidepressive agents in almost every psychological distress and negative mood disorder, disregarding other therapeutic options and without examining whether they are necessary or not. According to the 2020 World Health Organization data, approximately 264 million people of all ages worldwide use antidepressants as they suffer from depression[1].

Studies in recent years have emphasized the relationship between depression and the body's inflammatory response. Differences were observed in plasma concentrations of depressed patients, cytokine levels and the number of immune cells. A number of studies have focused on the direct and indirect effects of antidepressant drugs on the immune system. Chronic inflammation and immune system disorders are involved in the pathogenesis of depression. It has been observed that the nervous endocrine and immune systems are in mutual interaction in patients with depressive syndrome, and even chronic stress and chronic inflammation are frequently encountered in these patients. Under stress, the transmission of nerve signals from the cerebral cortex to the hypothalamus accelerates, and adrenocorticotropic hormone (ACTH) is secreted from the pituitary gland. Thus, glucocorticoids are released from the adrenal cortex. Glucocorticoids, on the other hand, suppress the production of cytokines that trigger the development of inflammation, such as interleukins (IL) and tumor necrosis factor α (TNFα), and also reduce the activity of T lymphocytes[2,3].

An increase in the number of monocytes, macrophages and neutrophils was observed in patients suffering from depression, and a reduce in the number of Natural Killer (NK) cells was observed[4,5]. It is thought that antidepressant drugs used in the treatment against such effects of depression on the immune system may have immunomodulatory properties in addition to their effects on neurotransmitter transporters and the neural membrane. In many studies, a decrease in serum cytokine levels has been found in patients using antidepressants[6,7].

For example, a meta-analysis over 20 years by Strawbridge showed that patients taking antidepressants had significantly reduced serum TNFα and interlukin-6 (IL-6) levels[8]. In some of the studies that found a decrease in the amount of serum cytokines, it was stated that this decrease was effective in placebo rather than antidepressant. Therefore, the net effects of antidepressants on the immune system have not been revealed[9,10]. Hydroxyzine hydrochloride is an antihistamine that is frequently preferred by individuals in recent years, and is widely used in the symptomatic treatment of anxiety disorder, mental distress and tension. It is also preferred in the treatment of allergies, as it blocks histamines in its mechanism of action. Histamines stimulate the release of cytokines in allergic inflammations. In 1997, Lagier et al. showed that histamines release IL-4 and Interferon-γ (IFN-γ) from T cells[11]. In addition, histamines inhibit monocyte product TNFα and IL-12, while inducing IL-10 and IL-18 synthesis[12-15]. Since hydroxyzine hydrochloride has antihistamine properties, the above effects should show the opposite. However, there have not been enough studies on the effects of hydroxyzine hydrochloride on the immune system. To further clarify these effects, the effects of hydroxyzine hydrochloride on TNFα, IL-6, IL12p40 and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) cytokine levels in the J774.2 macrophage cell line were investigated in this study.

Macrophages are one of the important cells of the natural immune system that play a role in the formation of inflammation[16-18]. TNFα plays an essential role in inflammatory

responses by producing proinflammatory cytokines such as IL1p, IL12, GM-CSF and IL6. They are concentrated in inflamed areas in chronic diseases and immune system disorders[19-22]. Macrophages have been the target of our study, with their antigen-presenting properties and their contribution to innate and acquired immunity and their important cytokine production role.

MATERIAL and METHODS

Hydroxyzine hydrochloride was purchased from Sigma Aldrich (CAS no: 2192-20-3) and used without further purification.

Cell Culture

J774.2 macrophage cells were obtained from ATCC. Cells were grown in Roswell Park Memorial Institute medium (RPMI 1640) containing 10% Fetal bovine serum (FBS), 1% antibiotics (100 µg/mL each penicillin and streptomycin) and sodium pyruvate. Cells were incubated at 37 °C in an incubator containing 5% CO2. The cell medium was renewed every four days to make it ready for use in the experiments[20].

Stimulation of lipopolisachharide (LPS) and hydroxyzine hydrochloride to mimic distress signal

J774.2 Macrophage cell was plated in 24-well plates at a cell concentration of 106 cells/mL per well. It was rested for 12 hours in an incubator with 5% CO2 at 37°C. The effect of hydroxyzine hydrochloride at 1-5-10 and 20 µg/mL concentrations alone and in wells with LPS added was tested. During these tests, salicylic acid, which proved to have anti-inflammatory properties, was put into one of the wells together with LPS and the additions were compared with other data. Also, Al2O3 was used with LPS to promote anti-inflammatory activity.

1 µl of LPS (1 mg/mL, Enzo Life Sciences, Salmonella minnesota R595) was added to J774.2 macrophage cell medium that was rested overnight. Macrophage cell medium. 1 µl of DMSO was placed in the control well and the control well containing LPS. The cells were then incubated for 24 hours in an incubator at 37 °C and 5% CO2.

Then, after centrifugation at 2000 Rpm, the supernatant was transferred to Eppendorf tubes. It was placed at -80°C to be examined in terms of cytokine levels. Cells were separated from each well by gentle pipetting. All experimental conditions were tested in triplicates, and all experiments were repeated 3 times in biological replicates to arrive at statistically significant data. To measure the level of TNFα, IL-6, IL12p40 and GM-CSF cytokine growth in J774.2 cells, 5 mM ATP (Fisher Scientific) was added to each well 2 hours before harvest[19-20].

TNFα, IL-6, IL12p40 and Gm-CSF ELISAs

Production of TNFα, IL-6, IL12p40 and GM-CSF was measured by enzyme-linked immunosorbent assay (ELISA). The ELISA kit for each cytokine type used in the study was prepared by following the BD Biosciences, CA, USA manufacturer's instructions exactly. First, the Maxisorb 96-well plate (Krackeler) was coated with hamster anti-mouse cytokine (bicarbonate buffer pH = 9.5, 0.5 µg/mL in 100 µL/well) and incubated overnight. After the solution was taken from the plates, they were washed 3 times with 0.05% Tween 20 PBS and incubated for 3 hours at room temperature. It was then blocked by adding 200 µL of blocking buffer (1% BSA PBS) to each well. Then the plates were washed 3 times and 100 µL of samples were placed in each well and incubated overnight at 4 °C. The plates were washed 3 times with 100 µL of biotin, then human anti-mouse cytokine (0.5 µg/mL in 10% FBS PBS) was added to each well and the plate was incubated for 2 hours at room temperature. Then the solutions were removed and the plate was washed 3 times. 100 µL of TMB substrate (BD OptEIA) was added to each well 19,20 The reaction was stopped using 50 µL of 1 M sulfuric acid and absorbance measurements were made at 450 nm. The concentration of TNFα, IL-6, IL12p40 and GM-CSF in each sample was calculated using known concentrations of each cytokine as a standard.

Statistical Analysis

GraphPad-Prism Software 5 program was used for statistical analysis and there were three independent results for each dataset, and an unpaired two-tailed t-test was used to reveal statistical significance. All experimental conditions tested in triplicate. $p < 0.0001$ N=3.

RESULTS

In the absence of the hydroxyzine hydrochloride LPS distress signal, no increase in TNFα levels was observed at all concentrations we used (1-5-10 and 20 μg/mL) (Figure 1). Hydroxyzine hydrochloride did not affect TNFα levels in macrophage cells after 24 hours of incubation and did not activate immune cells. There was no statistical difference in this value, which did not show an immunostimulatory effect. LPS positive controls induced TNFα production after 24 hours of incubation as expected (Figure 1). When salicylic acid, which is well known for its anti-inflammatory potential, and LPS were used together, a slight decrease in TNFα level was observed compared to using LPS alone. We observed that when AL2O3 and LPS are used together, it increases the inflammatory effect in terms of TNFα level compared to the effect of LPS alone. (Figure 1).

In the absence of LPS danger signal, no increase in IL-6 levels was observed at all concentrations (1-5-10 and 20 μg/mL) where we used hydroxyzine hydrochloride (Figure 2). There was no statistical difference in this value, which did not show an immunostimulatory effect. LPS positive controls induced IL-6 production after 24 hours of incubation as expected (Figure 2). The combination of salicylic acid and LPS, which is well known for its anti-inflammatory potential, resulted in some reduction in IL-6 level compared to using LPS alone. When AL2O3 and LPS were used together, it increased the inflammatory effect in terms of IL-6 level compared to the effect of LPS alone (Figure 2).

In the absence of LPS danger signal, no increase in GM-CSF levels was observed at all concentrations (1-5-10 and 20 μg/mL) where we used hydroxyzine hydrochloride did not activate (Figure 3). There was no statistical difference in this value, which did not show an immunostimulatory effect. LPS positive controls induced IL-6 production after 24 hours of incubation as expected (Figure 3). The combination of salicylic acid and LPS, which is well known for its anti-inflammatory potential, resulted in some reduction in the level of GM-CSF compared to using LPS alone. When AL2O3 and LPS were used together, it increased the inflammatory effect in terms of GM-CSF level compared to the effect of LPS alone (Figure 3).

In the absence of LPS danger signal, no increase in IL-12p40 levels was observed at all concentrations (1-5-10 and 20 μg/mL) where we used hydroxyzine hydrochloride (Figure 4). There was no statistical difference in this value, which did not show an immunostimulatory effect. LPS positive controls induced IL-6 production after 24 hours of incubation as expected (Figure 4). The combination of salicylic acid and LPS, which is well known for its anti-inflammatory potential, resulted in some reduction in IL-12p40 level compared to using LPS alone. When AL2O3 and LPS are used together, it increased the inflammatory effect in terms of IL-12p40 level compared to the effect of LPS alone (Figure 4).

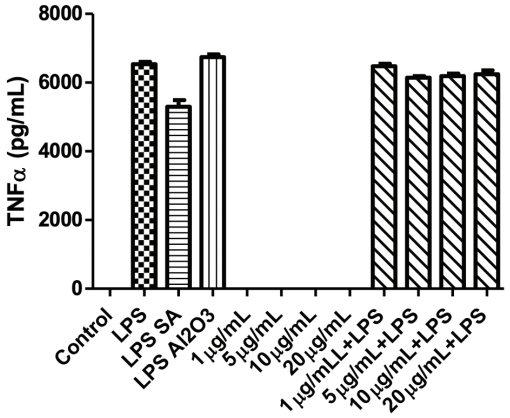

Figure 1. ELISAs for supernatants of TNFα macrophage cells were stimulated with 1 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL hydroxyzine hydrochloride for 24 hours. Cell concentration of 1 x 106 cells/mL and DMSO for negative control, 1μg/mL LPS for positive control and 1 μg/mL, 5 μg/mL,10 μg/mL and 20 μg/mL hydroxyzine hydrochloride without LPS dissolved in DMSO and DMSO were applied. All experimental conditions tested in triplicate. Student's t test was used for statistical analysis.

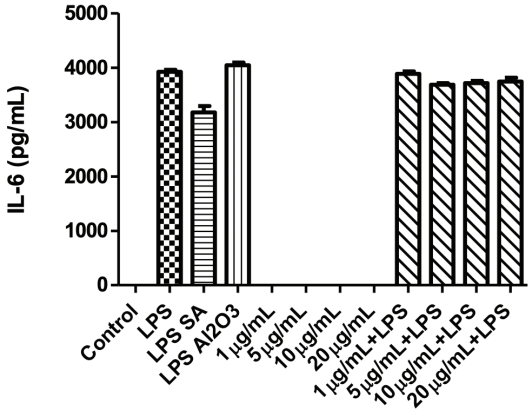

Figure 2. ELISAs for supernatants of IL-6 macrophage cells were stimulated with 1 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL hydroxyzine hydrochloride for 24 hours. Cell concentration of 1 x 106 cells/mL and DMSO for negative control, 1μg/mL LPS for positive control and 1 μg/mL, 5 μg/mL,10 μg/mL and 20 μg/mL hydroxyzine hydrochloride without LPS dissolved in DMSO and DMSO were applied. All experimental conditions tested in triplicate. Student's t test was used for statistical analysis.

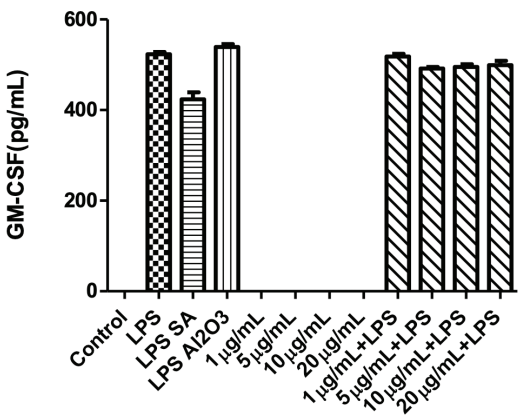

Figure 3. ELISAs for supernatants of GM-CSF macrophage cells were stimulated with 1 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL hydroxyzine hydrochloride for 24 hours. Cell concentration of 1 x 106 cells/mL and DMSO for negative control, 1μg/mL LPS for positive control and 1 μg/mL, 5 μg/mL,10 μg/mL and 20 μg/mL hydroxyzine hydrochloride without LPS dissolved in DMSO and DMSO were applied. All experimental conditions tested in triplicate. Student's t test was used for statistical analysis.

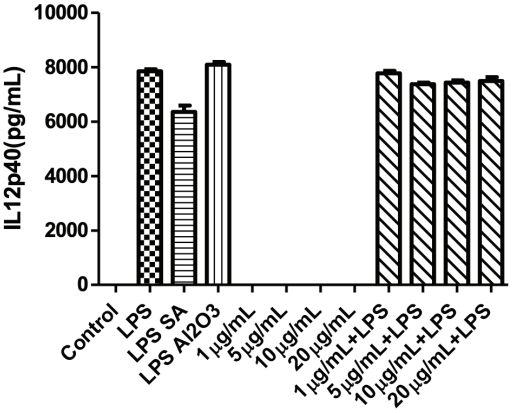

Figure 4. ELISAs for supernatants of IL12p40 macrophage cells were stimulated with 1 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL hydroxyzine hydrochloride for 24 hours. Cell concentration of 1 x 106 cells/mL and DMSO for negative control, 1μg/mL LPS for positive control and 1 μg/mL, 5 μg/mL,10 μg/mL and 20 μg/mL hydroxyzine hydrochloride without LPS dissolved in DMSO and DMSO were applied. All experimental conditions tested in triplicate. Student's t test was used for statistical analysis.

DISCUSSION

The majority of patients with psychological distress are directed to antidepressant use without trying other therapeutic treatments. The use of antidepressants worldwide has increased by 18% in the last ten years and continues to increase. It is a question of interest to what extent the effects of antidepressants, which are used so extensively, on the immune system, have been carried out in detail. Studies have investigated the effects of antidepressant classes on both the innate and adaptive immune systems. In this study, we observed the effects of hydroxyzine hydrochloride which is preferred in the treatment of depression and anxiety, on TNFα, IL-6, GM-CSF, L12p40 cytokine levels in macrophage cells. J774.2 Macrophage cells were exposed to 1-5-10-20 μg/mL hydroxyzine hydrochloride for 24 hours without the LPS distress signal and accompanied by the LPS distress signal. During these tests, salicylic acid, which proved to have anti-inflammatory properties, was put into one of the wells together with LPS and the additions were compared with other data. Also, Al2O3 was used with LPS to promote anti-inflammatory activity. As a result of our study, we did not observe any increase in the levels of proinflammatory cytokines TNFα, IL-6, GM-CSF, L12p40 of hydroxyzine hydrochloride. We observed that hydroxyzine hydrochloride did not have an immunostimulatory effect for macrophage cells and did not have positive or negative effects on the immune system in terms of inflammatory effects (Figure 1,2,3,4) Based on our results, hydroxyzine hydrochloride can be used safely in the treatment of depression. There is no previous study in the literature on the effect of hydroxyzine hydrochloride on the immune system. Therefore, we will compare the results of our study with those performed with other antidepressant drugs.

In the first study examining the immune system effect of antidepressants, Landmann et al. Immunity measurements were made from the serum of 22 patients and 22 healthy individuals using antidepressants, and no change was observed in monocyte activity. In subsequent studies, it has been shown that the immune effect of antidepressant drugs is often irregular in patients with depression[25]. Much of the work in psychoimmunology is concerned with the response of inflammation and depression to the innate immune response. In a meta-analysis of 24 studies by Dowloti et al., the pro-inflammatory cytokines TNFα and IL-6 were found in significantly higher concentrations in depressed individuals compared to controls[27]. In another meta-analysis that depression elicits an inflammatory response, IL-6, C-reactive protein (CRP) levels were found to be higher in depressed subjects compared to controls[28]. Studies and meta-analysis evaluations suggest that antidepressants may have effects on the immune system. A meta-analysis of 22 studies showed that selective serotonin reuptake inhibitor (SSRI) antidepressants can reduce TNFα and IL-6 levels compared to other antidepressants in 603 patients using antidepressants. However, studies proving this have not been done[29].

In studies examining the effects of antidepressant drugs in-vivo in mice, it has been shown that they especially affect the cytokine production of immune cells[30,31]. Comparing the blood monocytes of healthy people and patients with depression, it has been shown that antidepressants downregulate the release of proinflammatory cytokines and upregulate the release of anti-inflammatory ones[31,32].

The difference between these studies and our results is due to the use of antidepressant drugs with different active ingredients in each. Therefore, triggering the cytokine production in the immune cells or keeping the cytokine level constant for each of them differed. As a result, hydroxyzine hydrochloride drug does not activate TNFα, IL-6, Gm-CSF, IL-12 p40 cytokines in macrophage cells at 1-5-10-20 μg/mL. It can be used safely in the treatment of depression and anxiety.

Conflict of Interest

The authors declare that there is no conflicts of interest.

References

1. *https://www.businesswire.com/news/home/20210426005303/en/Global-Antidepressants-Market-Report-2021-COVID-19-Causes-a-Surge-in-Demand-for-Antidepressant-Drugs-as-Mental-Health-Problems-Rise-ResearchAndMarkets.com*
2. *Oppong E, Cato AC. Effects of glucocorticoids in the immune system. Glucocorticoid Signaling 2015; 52: 217-233.*
3. *Cubała, WJ, Landowski J, Dziadziuszko M, Chrzanowska A, Wielgomas B. Magnesium C-reactive protein, and cortisol in drug-naïve patients with short illness-duration, first episode major depressive disorder: possible immunomodulatory role for magnesium. Magnesium Research 2016; 24: 169-174.*
4. *Demir S, Atli A, Bulut M, İbiloğlu AO, Güneş M, Kaya MC, et al. Neutrophil–lymphocyte ratio in patients with major depressive disorder undergoing no pharmacological therapy. Neuropsychiatric Disease and Treatment 2015; 11: 2253.*
5. *Wei J, Zhang M, Zhou J. Myeloid-derived suppressor cells in major depression patients suppress T-cell responses through the production of reactive oxygen species. Psychiatry Res 2015; 228:695–701.*
6. *Hannestad J, Dellagioia N, Bloch M. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. Neuropsychopharmacology 2011; 36:2452–2459.*
7. *Dahl J, Ormstad H, Aass HCD, Malt UF, Bendz LT, Sandvik L, et al. The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. Psychoneuroendocrinology 2014;45:77-86.*
8. *Strawbridge R, Arnone D, Danese A, Papadopoulos A, Vives AH, Cleare AJ. Inflammation and clinical response to treatment in depression: a meta-analysis. European Neuropsychopharmacology 2015;25:1532-1543.*
9. *Brunoni AR, Machado-Vieira R, Sampaio-Junior B, Vieira EL, Valiengo L, Benseñor IM, Teixeir AL. Plasma levels of soluble TNF receptors 1 and 2 after tDCS and sertraline treatment in major depression: results from the SELECT-TDCS trial. Journal of Affective Disorders 2015;185:209-213.*
10. *Szalach LP, Lisowska KA, Cubala WJ. The influence of antidepressants on the immune system. Archivum Immunologiae et Therapiae Experimentalis 2019; 67:143-151.*
11. *Lagier B, Lebel B, Bousquet J, Pene J. Different modulation by histamine of IL-4 and interferon-gamma (IFN-γ) release according to the phenotype of human Th0, Th1 and Th2 clones. Clinical & Experimental Immunology 1997;108:545-551.*
12. *Vannier E, Miller LC, Dinarella CA. Histamine suppresses gene expression and synthesis of tumor necrosis factor alpha via histamine H2 receptors. J Exp Med 1991; 174:281-284.*
13. *Van der Pouw Kraan TC, Snijders A, Boeije LC, de Groot ER, Alewijnse AE, Leurs R, et al. Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. The Journal of Clinical Investigation 1998; 102:1866-1873.*
14. *Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. The Journal of Immunology 1998:161(5):2586-2593.*
15. *Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Critical Reviews in Eukaryotic Gene Expression 2010; 20: 87–103.*
16. *Lopez-Abente G, Mispireta S, Pollan M. Breast and prostate cancer: an analysis of common epidemiological features in mortality trends in Spain. BMC Cancer 2014;14(1): 1-10.*
17. *Ince MA, Hausmann MV, Martínez-Diaz DM. Guldi, Torres T. Non-covalent versus covalent donor-acceptor systems based on near-infrared absorbing azulenocyanines and C60 fullerene derivatives. Chemical Communications 2012; 48: 4058–4060.*
18. *Ayaz F. Ruthenium pyridyl thiocyanate complex increased the production of pro-inflammatory TNFα and IL1β cytokines by the LPS stimulated mammalian macrophages in vitro. Molecular Biology Reports 2018; 45(6):2307-2312.*
19. *Ayaz F, Yuzer A. Immunostimulatory effect of Zinc Phthalocyanine derivatives on macrophages based on the pro-inflammatory TNFα and IL1β cytokine production levels M. Ince. Toxicol. In Vitro 2018; 53: 172–177.*
20. *Önal HT, Yuzer A, Ince M, Ayaz F. Photo induced anti-inflammatory activities of a Thiophene substituted subphthalocyanine derivative. Photodiagnosis and Photodynamic Therapy 2020; 30:1-7.*
21. *Gasparini C, BM Foxwell, Feldmann M. RelB/p50 regulates TNF production in LPS-stimulated dendritic cells and macrophages. Cytokine 2013;61:736–740.*
22. *Jacobsen PL, Thorley EM, Curran C. Real-world patient experience with sexual dysfunction and antidepressant use in patients with self-reported depression: A cross-sectional survey study. Neurology, Psychiatry and Brain Research 2020; 36:57-64.*
23. *Landmann R, Schaub B, Link S, Wacker HR. Unaltered monocyte function in patients with major depression before and after three months of antidepressive therapy. Biol. Psychiatry 1997;41: 675–681.*
24. *Lee KM, Kim YK. The role of IL-12 and TGF-beta1 in the pathophysiology of major depressive disorder. Int. Immunopharmacol 2006;6:1298–1304.*
25. *Müller N, Hofschuster E, Ackenheil M, Mempel W, Eckstein R. Investigations of the cellular immunity during depression and the free interval: evidence for an immune activation in affective psychosis. Progress in Neuro-Psychopharmacology and Biological Psychiatry 1993;17:713-730.*
26. *Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimaki M. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. Brain Behavior and Immunity 2015; 49: 206-215.*
27. *Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biological Psychiatry 2010;67: 446-457.*
28. *Hannestad J, Dellagioia N, Bloch M. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. Neuropsychopharmacology 2011; 36: 2452-2459.*
29. *Kubera M, Curzytek K, Majewska-Szczepanik M, Szczepanik M, Marcinska K, Ptak W et al. Inhibitory effect of antidepressant drugs on contact hypersensitivity reaction. Pharmacological Report 2012; 64:714–722.*
30. *Curzytek K, Kubera M, Szczepanik M, Basta-Kaim A, Leskiewicz M, Budziszewska B et al. Crosstalk between contact hypersensitivity reaction and antidepressant drugs. Pharmacological Report 2013; 65: 1673–1680.*
31. *Lin A, Song C, Kenis G, Bosmans E, De Jongh R, Scharpe S, et al. The in vitro immunosuppressive effects of moclobemide in healthy volunteers. J. Affect. Disord 2000; 58: 69–74.*
32. *Xia Z, DePierre JW, Nassberger L. Tricyclic antidepressants inhibit IL-6, IL-1 beta and TNF-alpha release in human blood monocytes and IL-2 and interferon-gamma in T cells. Immunopharmacology 1996; 34: 27–37.*