Original Article

# Evaluation of the Effect of Ciprofloxacin on some Cytokines in Mice

**Aqeel H. Atallah[1], Nadia N. H. AL Masaoodi[2], Khamael H. Obaid[3]**
[1,2,3]College of Medical and Health Technologies, Al-Zahraa University for Women, Karbala 56100, Iraq
Email: aqeel.hayder@alzahraa.edu.iq
**DOI:** 10.47750/pnr.2022.13.S06.162

## Abstract

The purpose of this study is to see if therapy with Ciprofloxacin (80 mg/kg body weight) for 3, 7, or 14 days has any influence on the levels of (Cytokines - 2, 4, 6, 10) in albino mice. The albino mice were put into four equal groups for the investigation. G. I: - The animals in this group were not given any material (control). G. II: - The animals in this group were given ciprofloxacin (80 mg/100 g body weight) (Dosage one per day Orally) for three days. G. III: - The animals in this group were given ciprofloxacin (80 mg/100 g body weight) (Dosage one per day Orally) for seven days. G. IV: - For 14 days, animals in this group were given ciprofloxacin (80 mg/100 g body weight) (Dosage one per day Orally). The results show that ciprofloxacin greatly increased Cytokines - 10 (especially after 14 days of therapy) ($p<0.01$), as well as Cytokines - 2, 6 ($p<0.01$), whereas Cytokines - 4 greatly decreased ($P<0.05$).

**Keywords:** Ciprofloxacin, Cytokines - 2, Cytokines 4, Cytokines 6, Cytokines 10.

## INTRODUCTION

Cytokines are immunomodulatory proteins that cause a wide range of responses in cells and tissues. Within mammals, these cytokines make up a vast number of known immune "second messenger" molecules. Cytokines start a reaction by attaching to high affinity receptors on the surface of cells; they work in a paracrine or autocrine manner, rather than as an endocrine signal, as steroidal and amino acid-derived hormones do. The ligands involved, specific receptors expressed on the cell surface, and the signaling cascades that are initiated all influence how a cell reacts to these cytokines. During an immune response, cytokines regulate growth, differentiation, and activation [1].

Cytokines -2 is a kind of cytokine signaling molecule found in the immune system. It's a protein that controls the activity of immune-system-building white blood cells (leukocytes, or lymphocytes). Cytokines -2 is involved in the body's normal reaction to microbial infection as well as the distinction between "self" and "non-self." Lymphocytes express Cytokines -2 receptors, which mediate the actions of Cytokines -2. Cytokines -2 is required for T-cell proliferation, differentiation, and maturation into 'effector' T-cells. T-cells generally release cytokines-2 during an immunological response [2]. T-cell growth factor, also known as cytokines-2, plays a key function in T-cell biology and can stimulate T-cell-dependent immunological responses [3]. The release of Cytokines -2 and the expression of Cytokines -2 receptors are stimulated when antigen binds to the T-cell receptor. The interaction of the Cytokines -2/Cytokines -2 receptors stimulates the growth, differentiation, and survival of antigen-specific CD4 + T-cells and CD8 + T-cells, implying that Cytokines -2 is required for the development of T-cell immunologic memory, which relies on the expansion of the number and function of antigen-selected T-cell clones [4].

Cytokines -4 (Interleukin-4, abbreviated (IL-4)) is a cytokine that causes naïve helper T-cells (Th0 cells) to differentiate into Th2 cells. Th2 cells create more Cytokines -4 after being activated by Cytokines -4. Although the cell that generates Cytokines -4, which induces Th0 differentiation, has yet to be identified, current research suggests that basophils might be the effector cell [5]. Cytokines -4 has a variety of biological functions, including promoting the proliferation of activated B- and T-cells and the differentiation of B-cells into plasma cells. It plays an important role in both humoral and adaptive immunity. B-cell class flipping to IgE is induced by Cytokines -4, which also increases MHC class II synthesis. Th1 cells, macrophages, IFN-gamma, and dendritic cell IL-12 production are all reduced by cytokines -4 [6].

Cytokines -6 (IL-6) is a cytokine that may function as both a pro-inflammatory and anti-inflammatory cytokine. The Cytokines -6 gene encodes it in humans [7]. T-cells and macrophages release cytokines -6 to promote immunological responses, such as during infection and after trauma, particularly burns or other tissue damage that causes inflammation. Cytokines -6 is also involved in the battle against infection, since it has been found in mice to be necessary for resistance to the bacteria Streptococcus pneumoniae [8]. In addition, osteoblasts release Cytokines -6, which stimulates the production of osteoclasts. Cytokines -6 is a pro-inflammatory cytokine produced by smooth muscle cells in the tunica media of numerous blood arteries. Cytokines -6 functions as an anti-inflammatory cytokine by inhibiting TNF-alpha and Cytokines -1, as well as activating Cytokines -1ra and Cytokines -10. Fever and the acute phase response are both mediated by cytokines -6. It has the ability to pass across the blood-brain barrier [9]. Cytokines -6 increases energy mobilization in muscle and fatty tissue, resulting in a rise in body temperature. Macrophages can produce cytokines -6 in response to certain microbial compounds known as pathogen-associated molecular patterns. These pathogen-associated molecular patterns connect to a class of innate immune system detecting molecules known as pattern recognition receptors, which includes Toll-like receptors. These are found on the cell surface and within the cell, and they trigger intracellular signaling cascades that lead to the generation of inflammatory cytokines. Cytokines -6 is sometimes referred to as a myokine, which is a cytokine generated by muscle and is increased in response to muscular contraction [10]. As a myokine, cytokines -6 possesses a wide range of anti-inflammatory properties. The first myokine discovered to be produced into the bloodstream in response to muscle contractions is cytokines -6 [11]. In many disorders, such as diabetes [12], depression [13], and rheumatoid arthritis [14], cytokines -6 increase the inflammatory and auto-immune processes.

Many cell populations generate Cytokines -10 (Interleukin -10), a key immune regulating cytokine. Its major biological role appears to be the control of development and proliferation of immune cells such as T-cells, B-cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes, as well as the limiting and termination of inflammatory reactions. Recent evidence suggests, however, that Cytokines -10 has immune-stimulatory qualities that aid in the elimination of infectious and noninfectious particles with minimal inflammation. Numerous studies, including patient expression assessments, in vitro and animal trials, reveal that Cytokines -10 has a vital role in inflammatory, neoplastic, and autoimmune illnesses. Overexpression of Cytokines -10 has been observed in cancers such as melanoma and various lymphomas, and it is thought to accelerate tumor growth. Following acute stress reactions, systemic Cytokines -10 release is a potent instrument of the central nervous system for preventing hyper inflammatory processes by activating the neuro-endocrine axis. In certain inflammatory illnesses defined by a type 1 cytokine pattern, such as psoriasis, a relative Cytokines -10 shortage has been reported and is thought to be of pathophysiological consequence. Human Cytokines -10 recombinant has been developed and is now being investigated in clinical studies. Rheumatoid arthritis, inflammatory bowel illness, psoriasis, organ transplantation, and chronic hepatitis C are among these conditions. The outcomes are a mixed bag. They add to our understanding of Cytokines -10 immunobiology and indicate that the Cytokines -10/Cytokines -10 receptor system might be a potential therapeutic target [15].

Ciprofloxacin is a fluoroquinolone antibiotic that may be used for a variety of infections. It has a high oral absorption and is used to treat a range of bacterial infections [16]. It also has antibacterial properties against Gram-positive and Gram-negative bacteria and is used to treat anthrax and plague infections. Adults should only use Ciprofloxacin extended-release pills. Liver damage is one of the worst side effects linked with its usage [17-18]. The most common symptom of ciprofloxacin-induced liver damage is an asymptomatic rise in liver enzymes. In rare situations, it can also cause acute hepatitis. We describe a case of acute hepatitis that occurred after using ciprofloxacin for a short period of time. It is given orally because it is rapidly absorbed, penetrates well into tissues, and inhibits DNA gyrase, which is equal to mammalian topoisomerase II [18]. Its antibacterial activity is mostly produced by inhibition of DNA gyrase, which is equivalent to mammalian topoisomerase II. Several biochemical, clinical, and epidemiologic investigations have found that ciprofloxacin therapy can result in significant liver failure. Furthermore, convulsions caused by ciprofloxacin have been a source of worry [17]. Despite the rarity of these adverse effects, the high prescription rates for these antibiotics may have serious health implications for the general populace. The purpose of this research was to learn more about ciprofloxacin's side effects.

## MATERIALS AND METHODS

Animals

A total of 150 mature female and male mice, ranging in age from 8 to 12 weeks and weighing 22 to 28 grams, were obtained from Iraq's Ministry of Health's National Center for Drug Control and Research. At Nahrain University's Biotechnology Research Center, they were maintained in a plastic box with hard wood chips for bedding in a controlled animal habitat at 25 C with a 4/10-hour light/dark cycle.

Drug

The antibiotics were administered orally three times a day for three, seven, and fourteen days. Ciprofloxacin (Cipro) ($C_{17}H_{18}FN_3O_3$•HCl•$H_2O$), ciprofloxacin hydrochloride tablets, Bayer healthcare pharmaceuticals The specified doses are equivalent to the human therapeutic amount, according to Guidance for Industry and Reviewers (2002) computations [19].

Experimental Design:

The trials were aimed to see how ciprofloxacin affected Cytokines - 2, 4, 6, 10 in albino mice

Group I: - The animals in this group were not given any material (control).

Group II: - The animals in this group were given ciprofloxacin (80 mg/100 g body weight) (Dosage one per day Orally) for three days.

Group III: - The animals in this group were given ciprofloxacin (80 mg/100 g body weight) (Dosage one per day Orally) for seven days.

Group IV: - For 14 days, animals in this group were given ciprofloxacin (80 mg/100 g body weight) (Dosage one per day Orally).

Serum Profile Assay

One ml of blood was taken from each animal through heart puncture using disposable small (insulin) needles (1 ml) inserted in an Eppendorf tube and allowed to clot at room temperature for roughly 10 minutes before centrifugation at 4000 rpm for 10 minutes to extract serum for further use.

Measurement of Serum Cytokines -2

A quantitative sandwich immunoassay is used in the Mouse Cytokines -2 (IL-2) enzyme-linked immunosorbent test (ELISA) (Catalogue Number: MEC1006). A monoclonal antibody specific for mouse Cytokines -2 was pre-coated on the microtiter plate included in this kit. After that, the relevant microtiter plate wells were filled with standards or samples, and they were incubated. If Mouse Cytokines -2 is present, the antibody pre-coated on the wells will attach to it and immobilize it.

The amount of mouse Cytokines -2 present in the standards/samples determines the level of color change.

Measurement of Serum Interleukin-4

Cytokines -4 in mice (Interleukin-4) The quantitative sandwich enzyme immunoassay method is used in the ELISA Assay Kit (Catalog Number: Cytokines 411-K01). A microplate has been pre-coated with a monoclonal antibody specific for Cytokines -4. Standards and samples are pipetted into the wells, and any Cytokines -4 present is bound by the immobilized antibody, followed by the addition of a detection antibody specific for Cytokines -4 to the wells, which binds to the combination of capture antibody Cytokines -4 in the sample. The amount of Cytokines -4 present in the sample determines the color of the product.

Measurement of Serum Cytokines -6

The Cytokines -6 (Interleukin-6) ELISA kit for mice is used to detect Cytokines -6 in plasma, serum, and cell culture materials (Catalog No. EMI1006-1). In less than 5 hours, this test uses a quantitative sandwich enzyme immunoassay method to detect Cytokines -6. A mouse Cytokines -6 specific rat monoclonal antibody has been pre-coated onto a 96-well microplate with detachable strips. The immobilized antibody specific for mouse Cytokines -6 is sandwiched between Cytokines -6 in standards and samples. After washing away any loose material, a peroxidase enzyme substrate is introduced. The color development is halted, and the color intensity is measured.

Measurement of Serum Cytokines -10

The Mouse Cytokines -10 ELISA kit detects mouse Cytokines -10 in mouse plasma, serum, tissue extracts, and cell culture materials (Catalog No. EMI3010-1). In less than 5 hours, this test evaluates mouse Cytokines -10 using a quantitative sandwich enzyme immunoassay method. A 96-well microplate with detachable strips has been pre-coated with a polyclonal antibody specific for mouse Cytokines -10. The immobilized antibody specific for mouse Cytokines -10 is sandwiched between Cytokines -10 in standards and samples. After washing away any loose material, a peroxidase enzyme substrate is introduced. The color development is halted, and the color intensity is measured.

Statistical Analysis

IBM's statistical software is used to examine the data (SPSS version 20). The principles of the considered parameters were presented in terms of Mean standard error, and analysis of variance was used to compare the means of all parameters (ANOVA). At $p<0.05$, differences were judged statistically significant.

## Results

Table (1-1) shows the results of the current investigation. When treated animals with Ciprofloxacin, the level of Cytokines - 6, 10 increased significantly ($p \leq 0.01$), it was (209, 215, and 226) respectively at 3, 7, and 14 days in comparison to control (201) and also Cytokines - 10 (374, 423, and 508) respectively at 3, 7, and 14 days in comparison to control (94) while Cytokines -4 decreased significantly (p0.01) at 3, 7, and 14 days, it was (113, 73, and (134). Furthermore, when the mice were given Ciprofloxacin for 14 days, the level of Cytokines -2 was considerably (p 0.01) higher (32) than in the control group (20).

Table (1-1) shows the effect of Ciprofloxacin on Cytokines - 2, 4, 6, 10

| **Parameter** | **Control** | **Ciprofloxacin** | | |
|---|---|---|---|---|
| | | **3 days (Mean ± SE)** | **7 days (Mean ± SE)** | **14 days (Mean ± SE)** |
| **Cytokines -2** | **20.3 ± 0.88** | **23 ± 1.15** | **27.3 ± 1.45** | **32 ± 2.3 **** |
| **Cytokines -4** | **134.6 ± 1.45** | **113.6 ± 2.60 **** | **73.3 ± 2.02 **** | **65.3 ± 1.76 **** |
| **Cytokines-6** | **201 ± 2.08** | **209.3 ± 1.45 *** | **215.6 ± 1.76 **** | **226 ± 1 **** |
| **Cytokines-10** | **94.3 ± 2.33** | **374.6 ± 12.97 **** | **423 ± 5.13 **** | **508 ± 28.69 **** |

refer to a significant * ($p \leq 0.05$) and ** ($p \leq 0.01$) differences between sample with control.

## Discussion

Ciprofloxacin was widely used to treat a variety of bacterial illnesses. Ciprofloxacin has antibacterial effects as well as promotion of hematopoiesis through increased IL-3 and inflammation reduction via IL-1, IL-6, and TNF- [20]. In contrast, Bailly et al. [21] discovered that ciprofloxacin therapy improves the ability of stimulated human monocytes to generate IL-6 in vivo in humans.

The current study supports a previous study that found a considerable increase in interleukin-2 production [22]. Fluoroquinolones may have immunomodulatory effects via changing the cytokine production of activated T cells, according to growing data [23].

Purswani et al. [24], who discovered that ciprofloxacin may reduce endotoxin-mediated mortality in mice and change early host cytokine responses, previously demonstrated the positive benefits of in vivo therapy with ciprofloxacin.

Ciprofloxacin is a highly useful antimicrobial since it affects a wide range of organisms [25], and [26] discovered that it has the potential to cause hepatotoxicity and other organ toxicity.

## Conclusion

According to the findings of this study, ciprofloxacin treatment increases cytokine levels, which has negative health consequences and is linked to immunomodulating and other hazardous disorders. Furthermore, ciprofloxacin's effects were more effective on immune cells that had been changed by ciprofloxacin-induced toxicity.

More descriptive research is needed to examine the harmful effects of ciprofloxacin on various organs and to learn about the chemicals that lower that toxicity, which will be beneficial to both animals and humans and useful against this antibiotic.

## REFERENCES


[1] Commins, S.P.; Borish, L. and Steinke, J.W. (2010). 'Immunologic messenger molecules: Cytokines, Interferons, and Chemokines', J. Allergy Clin. Immunol. Vol. 125, pp. S53–S72.
[2] Cantrell, D.A. and Smith, K.A. (1984). "The interleukin-2 T-cell system: a new cell growth model". Science, 224 (4655): 1312-6.
[3] Crispin, J.C. and Tsokos, G.C. (2008). Novel molecular targets in the treatment of systemic lupus erythematosus. Autoimmune Rev, 7: 256-261.
[4] Beadling, C. and Smith, K.A. (2002). "DNA Array Analysis of Interleukin-2-Regulated Immediate/Early Genes". Med Immunol 1 (1): 2.
[5] Sokol, C.L.; Barton, G.M.; Farr, A.G. and Medzhitov, R. (2008). "A mechanism for the initiation of allergen-induced T helper type 2 responses". Nat. Immunol. 9 (3): 310-318.
[6] Guo, N.; Xie, Z. and Shen, B. (2005). The development of new formats of Engineered Bispecific Antibodies: Trends in Immunology Research. pp.33-54
[7] Ferguson-Smith, A.C.; Chen, Y.F.; Newman, M.S.; May, L.T.; Sehgal, P.B. and Ruddle, F.H. (1988). "Regional localization of the interferon-beta 2/B-cell stimulatory factor 2/hepatocyte stimulating factor gene to human chromosome 7p15-p21". Genomics 2 (3): 203–208.
[8] Van der Poll, T.; Keogh, C.V.; Guirao, X.; Buurman, W.A.; Kopf, M. and Lowry, S.F. (1997). "Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia". J. Infect. Dis., 176 (2): 439-444.
[9] Banks, W.A.; Kastin, A.J. and Gutierrez, E.G. (1994). "Penetration of interleukin-6 across the murine blood–brain barrier". Neurosci. Lett. 179 (1-2): 53-6.
[10] Febbraio M.A, Pedersen B.K (2005). Contraction-induced myokine production and release: is skeletal muscle an endocrine organ?. Exerc. Sport. Sci. Rev. 33(3):114-119.
[11] Pedersen, B.K. and Febbraio, M.A. (2008). "Muscle as an endocrine organ: focus on muscle-derived interleukin-6". Physiol. Rev. 88 (4): 1379-406.
[12] Kristiansen, O.P. and Mandrup-Poulsen, T. (2005). "Interleukin-6 and diabetes: the good, the bad, or the indifferent?". Diabetes. 54 Suppl 2: S114-24.
[13] Dowlati, Y.; Herrmann, N.; Swardfager, W.; Liu, H.; Sham, L.; Reim, EK. and Lanctot, K.L. (2010). "A meta-analysis of cytokines in major depression". Biological Psychiatry, 67 (5): 446-457.
[14] Nishimoto, N. (2006). "Interleukin-6 in rheumatoid arthritis". Curr Opin Rheumatol 18 (3): 277-281.
[15] Asadullah, K.; Sterry, W. and Volk, H.D. (2003). Interleukin-10 Therapy Review of a New Approach, 55(2):241-269.
[16] The safety profile of the fluoroquinolones. Bertino J Jr, Fish D. Clin Ther. 2000; 22:798–817.
[17] Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. Chalasani N, Fontana RJ, Bonkovsky HL, Watkins PB, Davern T, Serrano J, Yang H, Rochon J; Drug Induced Liver Injury Network (DILIN) Gastroenterology. 2008; 135:1924–1934.
[18] Drug-induced liver disease. Zimmerman HJ. Clin Liver Dis. 2000; 4:73–96.
[19]- Guidance for Industry and Reviewers (2002). Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. Food and Drug Administration.
[20] Dalhoff A, Shalit I (2003) Immunomodulatory effects of quinolones. Lancet Infect Dis 3: 359–371.
[21] Bailly S., Fay M., Ferrua B., Gougerot-Pocidalo M.A.: Ciprofloxacin treatment in vivo increases the ex vivo capacity of lipopolysaccharide-stimulated human monocytes to produce IL-1, IL-6 and tumour necrosis factor-alpha. Clin Exp Immunol 1991, 85, 331–334.
[22] ZEHAVI-WILLNER T. & I. SHALIT I. Enhancement of interleukin-2 production in human lymphocytes by two new quinolones derivatives. Lymphokine Res. 8:35-46. (1989)
[23] Dalhoff A, Shalit I (2003) Immunomodulatory effects of quinolones. Lancet Infect Dis 3: 359–371.
[24] Purswani M.U., Eckert S.J., Arora H.K., Moel G.J.: Effect of ciprofloxacin on lethal and sublethal challenge with endotoxin and on early cytokine response in a murine in vivo model. J Antimicrob Chemother 2002, 50, 51–58.
[25] - Dana EK, Robb M, Sandra HL (2001) New classification and update on the quinolone antibiotics. Amer. Fam. Physic.
[26] - Adikwu E, Brambaifam N (2012) Ciprofloxacin cardio- toxicity and hepatoxicity in human and animals. Pharmcol. 3: 207-213.