Scientific Reports

https://doi.org/10.1038/s41598-025-34764-z

Article in Press

Effect of ciprofloxacin and vitamin C on immunotoxicity biomarkers and mesenteric lymph nodes in a rat model

Received: 9 February 2025

Accepted: 31 December 2025

Published online: 07 January 2026

Cite this article as: Nusair S.D., Abuu Ganem H.J., Al-Essa K. *et al.* Effect of ciprofloxacin and vitamin C on immunotoxicity biomarkers and mesenteric lymph nodes in a rat model. *Sci Rep* (2026). https://doi.org/10.1038/s41598-025-34764-z

Shreen Deeb Nusair, Haneen J. Abuu Ganem, Khansaa Al-Essa, O'la Ahmad AL-Fawares, Khawla Nuseir & Nehad M. Ayoub

Effect of Ciprofloxacin and Vitamin C on Immunotoxicity Biomarkers and Mesenteric Lymph Nodes in a Rat Model

Shreen Deeb Nusair [a]*, Haneen Abuu Ganem [b], Khansaa Al-Essa [c], O'la Ahmad AL-Fawares [d], Khawla Nuseir [a], Nehad M. Ayoub [a]

[a] Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan

[b] Department of Forensic Medicine, Toxicology and Forensic Sciences, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

[c] Department of Chemistry, Faculty of Science, Jerash University, Jerash, Jordan

[d] Department of Applied Biological Sciences, Faculty of Science, Al-Balqa Applied University, AL-Salt, Jordan

* **Corresponding author**: Shreen Deeb Nusair. Email: sdnusair@just.edu.jo; Tel. + 962 (0) 2 720 1000 ext. 26173; ORCID ID#: 0000-0001-7209-6249

Word count including references, figures and captions: 5079

Abbreviations: Cipro, Ciprofloxacin; VitC, vitamin C; FDA, Food and Drug Administration; EMA, European Medicine Agency; IL-4, interleukin-4; IL-10, interleukin-10; INF-ɣ, interferon-gamma;

ROS, reactive oxygen species; DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms; ACUC, Animal Care and Use Committee; ELISA, enzyme-linked immunosorbent assay; pg, picogram; ANOVA, analysis of variance; P value, significance value; tingible body macrophage, TBMs; Th2, T helper 2; PHA, physapruin A.

Abstract

Ciprofloxacin (Cipro) has been associated with various adverse effects, including potential toxicity to the immune system. This study investigates the immunotoxic effects of Cipro and examines the possible protective role of vitamin C (VitC) using rats. Although rats can synthesize VitC, they serve as a relevant model for evaluating drug effects and immune responses due to their physiological and immunological similarities to humans. A total of 40 rats were divided into four groups (n = 10). The Cipro group received 750 mg/kg of Cipro orally each day for five days. The VitC-Cipro group was administered 200 mg/kg of VitC orally one hour prior to receiving Cipro daily for five days. The VitC group received the same dose of VitC, while the Control group was given 0.9% normal saline daily for five days. Levels of interleukin-4 (IL-4), interleukin-10 (IL-10), and interferon-gamma (INF-γ) were measured in serum. Histopathological examinations of mesenteric lymph nodes were also conducted. The Cipro group exhibited significantly lower levels of IL-4 compared to all other groups ($P < 0.05$). Similarly, INF-γ levels were significantly reduced in the Cipro group compared to the VitC-Cipro group ($P < 0.05$) and the Control group ($P < 0.01$). No significant differences in IL-10 levels were observed among the groups ($P > 0.05$). Histological analysis revealed degenerative changes in the lymph nodes of the Cipro group, characterized by a moth-eaten appearance, tingible body macrophages, localized hemorrhage, and reactive eosinophilia. Tissue sections from the VitC-Cipro group showed a reduced number of

lymphocytes with uneven distribution in the paracortex reticular tissue. These alterations in interleukin levels and lymph node histology may indicate ciprofloxacin-induced immunotoxicity. Furthermore, the ameliorative effects of VitC against Cipro toxicity suggest its potential utility in cases of antibiotic overdose.

Keywords

Ciprofloxacin; Interleukin-4; Interleukin-10; Interferon-gamma; Mesenteric lymph nodes; Immunotoxicity; Biomarkers

1. Introduction

Ciprofloxacin (Cipro) is a broad-spectrum synthetic antimicrobial agent belonging to the second generation of fluoroquinolones. It exerts its antibacterial effects by inhibiting DNA gyrase and topoisomerase IV, critical enzymes involved in DNA replication [1]. Cipro's high bioavailability and rapid absorption, particularly its penetration into cerebrospinal fluid, contribute to its widespread clinical use [2].

Despite its efficacy, Cipro can induce toxicity, particularly with overdose or prolonged use. The mechanisms underlying fluoroquinolone toxicity include oxidative stress and the generation of reactive oxygen species (ROS), as well as nitrosative stress [3]. These free radicals can lead to various adverse effects, prompting restrictions on Cipro use during pregnancy, lactation, and in pediatric and elderly populations [4].

Cipro is associated with several adverse effects, ranging from localized reactions to severe systemic conditions. For instance, Toxic epidermal necrolysis (TEN), a severe cutaneous reaction characterized by extensive epidermal detachment and a high mortality rate, has been reported following ciprofloxacin administration [5]. Peri-orbital edema, along with itching and redness, has been observed in patients using ciprofloxacin eye drops [5]. Fluoroquinolones, including ciprofloxacin, can induce toxic effects on peripheral nerves, potentially leading to peripheral neuropathy, characterized by symptoms such as pain, burning, tingling, numbness, or

weakness [6]. Furthermore, Cipro has been associated with cardiotoxicity and hepatotoxicity. Ciprofloxacin can cause Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) syndrome, a severe hypersensitivity reaction involving multiple organ systems [7].

Food and Drug Administration (FDA) and the European Medicine Agency (EMA) have advised caution regarding fluoroquinolone prescriptions, particularly for uncomplicated infections, emphasizing the need for careful risk-benefit assessments [8].

Antioxidants like Vitamin C have shown potential in mitigating Cipro-induced toxicities. Studies indicate that Vitamin C can protect against ROS generation and phototoxicity associated with fluoroquinolones [9]. However, caution is warranted, as concurrent use of Vitamin C may reduce Cipro's antibacterial activity, necessitating careful adjustment of administration protocols [10].

In the context of assessing immunotoxicity biomarkers, a recent study explored the protective role of VitC against Cipro-induced immunotoxicity. Systemic immune parameters, including IL-4, IL-10, and IFN-ɣ, were measured in Cipro-overdosed rats receiving VitC as a prophylactic agent. Local immune responses were evaluated through histopathological examination of mesenteric lymph nodes. Comparisons were made with three groups receiving Cipro only, VitC only, and Control receiving saline only.

The rationale for using rats in this research is their physiological similarities to humans, which facilitate relevant extrapolation of findings to human health [11]. While rats synthesize Vitamin C, their immune responses are comparable to those in humans, enhancing the clinical relevance of the results [12]. In contrast, guinea pigs, which cannot synthesize vitamin C [13], may present different physiological responses to supplementation and drug interactions [14].

2. Materials and Methods

2.1. Ethical Use of Animals

Experiments on animals were designed and conducted according to the National Guide for the Animal Care and Use of Laboratory Animals, which were according to the Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology (Approval No. 753/2/3/16). All mandatory laboratory health and safety procedures have been complied within the course of conducting any experimental work reported herein. This study is reported in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) for reporting animal research.

2.2. Animal Conditions

A total of 40 adult male Sprague-Dawley rats (10-11 weeks, 174-190 g body weight) were housed in the Animal Care Unit at Jordan University of Science and Technology. The animals were examined by an assigned veterinarian. Rats were randomly assigned to one of four groups (n=10) by

a technician not otherwise involved in the study. Animals had a week for acclimatization in a stainless-steel wire cage (L x W x H; 37 x 22 x 25 cm^3), on a bedding of hardwood chips with free access to water and standard chow [15]. The temperature was controlled around 25 ± 2 ^oC at a 26% humidity and a 12-hour light: dark cycle [16].

2.3. Cipro and VitC Administration Protocols

Pre-treatment protocol (treatment before intoxication) was employed for the *in vivo* experiments to simulate the real practical clinical use of prophylaxis, and to show the ameliorative efficacy of VitC against cipro immunotoxicity. The groups of rats were treated as follows; the Cipro group had oral dose of 750 mg/kg cipro once daily for 5 days. The VitC-Cipro group had orally 200 mg/kg VitC one hour before the cipro overdose. The VitC group had only VitC, while the Control group had 0.9% normal saline solution. All doses were prepared freshly in 0.9% normal saline solution before oral administration and were given once daily for 5 days. The doses were according to previous studies on Cipro [17], and VitC [18]. During all experiments, animals were monitored daily, particularly for one hour following each dose [19]. No clinical or behavioral changes were detected.

2.4. Measuring IL-4, IL-10 and INF-ɣ Using ELISA

Blood samples (2 ml) were taken retro-orbital at the end of the experiment. The immune biomarkers IL-4, IL-10 and INF-ɣ were measured using enzyme linked immunosorbent assay (ELISA) [15]. The analysis was performed by

an investigator blinded to the group allocations. The samples were collected in golden-jell cap tubes, left 30 minutes at room temperature to coagulate. Later, the samples were cold centrifuged (4 ºC) at 3,000 x g for 10 min in a K3 series refrigerated centrifuge (Centurion Scientific, UK), and finally stored in aliquots at – 20 ºC for later analysis. The assay was performed using Rat IL-4 ELISA kit (ab100770, Abcam, USA), Rat IL-10 ELISA kit (ab100764, Abcam, USA), and Rat INF-ɣ ELISA Kit (ab46107, Abcam, USA). To optimize serum dilutions, a pilot run was conducted for the three kits using 1:1, 1:2, and 1:4 sample dilutions. Based on this, the dilution of 1:2 was selected for the three assays. The assays were conducted according to the manufacturers' instructions, except two modifications for measuring IL-4, where incubation time of samples was adjusted to 3 hours instead of 2 hours, while for the substrate, time was duplicated to 20 minutes instead of 10 minutes. Samples were processed in duplicates and measured at 450 nm wavelength using a microplate reader, model 1510 Multiskan Go Spectrophotometer (Thermo Fisher, UK).

2.5. Pathohistological Examination

By the end of the experiment, a brief sedation with 3% isoflurane was performed right before euthanasia by decapitation. The mesenteric lymph nodes from two rats per group were preserved in 10% formalin. Then tissues were cut into 0.5 µm sections using a rotary microtome of the model HM 325 (Thermo Scientific, USA). Processing of sections was performed

according to a previously described protocol [20]. Processed sections were placed on glass slides and stained using Hematoxylin-Eosin stain (H&E). Slides were left to air dry then were examined under a digital light microscope, model B-290TB (Optika, Italy). It is worth noting that while H&E stain is useful for identifying overall lymph node structural changes and inflammation, Immunohistochemical Staining is recommended to specifically identify T cells, B cells, and macrophages. In addition, future molecular studies can identify the alterations in the interleukin genes and verify their serum levels.

2.6. Statistical Analysis of Data

The statistical analysis was conducted by a researcher blinded to the experimental groups. All data are expressed in mean ± standard error (M ± SE). The data were analyzed using ANOVA in the GraphPad Prism version 8.0.1 (GraphPad Software, USA, 2018). Tukey's contrast analysis to determine any significance between and within groups. Shapiro-Wilk test was conducted to check the normality of data. The level of significance was set to the value $P < 0.05$ with confidence interval (CI) of 95%. ImageJ 1.53K software (National Institute of Health, USA, 2021) was used to analyze the cell count in the mesenteric lymph nodes sections form each animal group [21].

3. Results

3.1. Measurements of Serum IL-4, IL-10 and INF-ɣ

The average concentration of serum IL-4 was 1.83 ± 0.42 pg/ml in the cipro overdosed rats; 3.63 ± 0.14 pg/ml in the rats that had VitC one hour before the cipro overdose; 3.48 ± 0.65 pg/ml in rats treated with VitC only; and 3.51 ± 0.02 pg/ml in control rats that had the normal saline. Overdosed rats that did not have Vit C recorded significantly 52.6% lower levels of IL-4 than rats of other groups ($P < 0.05$). **Fig.1** shows the serum levels of IL-4 in the challenged rats.

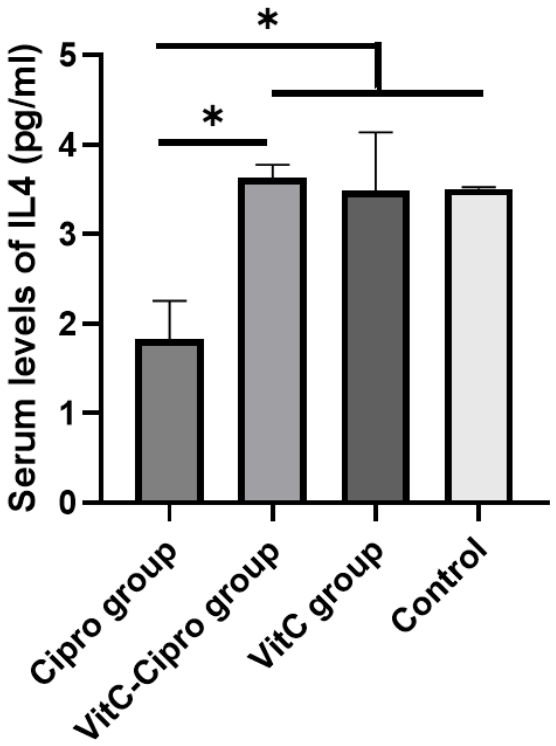

Fig.1 Mean concentrations (pg/ml) of IL-4 in serum of 4 groups of rats. From left to right: a group had 750 mg/kg cipro; a group had 200 mg/kg VitC then 1 hour later 750 mg/kg cipro; a group had VitC; and

control had normal saline. All doses were orally given once daily for 5 days. Whiskers are representing standard errors

The average serum levels of IL-10 were 59 ± 12.64 pg/ml in the overdosed rats; 58.65 ± 6.43 pg/ml in the rats with VitC prophylaxis; 63.95 ± 6.57 pg/ml in the VitC group; and 68.41 ±9.90 pg/ml in control. No significant changes ($P > 0.05$) were found between all groups with a 13% decrease in the levels of Cipro intoxicated rats relative to the control. **Fig.2** depicts serum levels of IL-10 in the four groups.

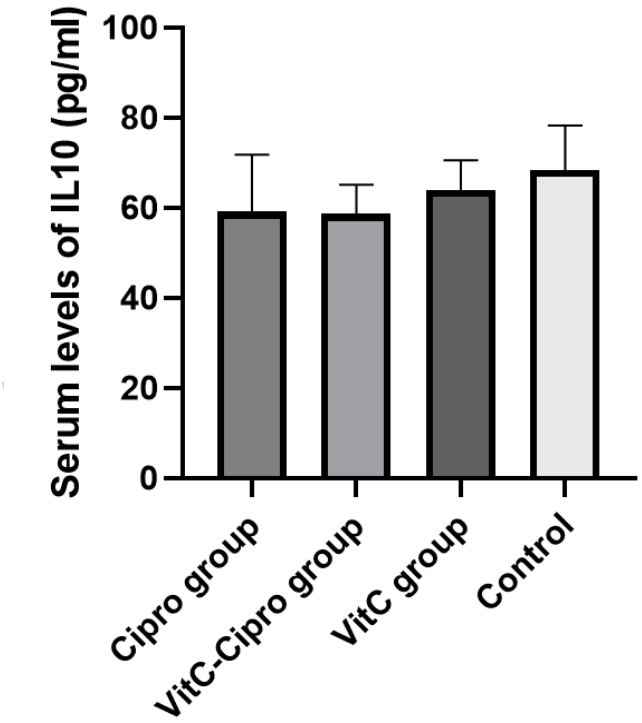

Fig.2 Mean concentrations (pg/ml) of IL-10 in serum of 4 groups of rats. From left to right: a group had 750 mg/kg cipro; a group had 200 mg/kg VitC then 1 hour later 750 mg/kg cipro; a group had VitC; and

control had normal saline. All doses were orally given once daily for 5 days. Whiskers are representing standard errors

The mean levels of serum INF-ɣ was 15.33 ± 4.21 pg/ml in the cipro intoxicated rats; 35.25 ± 7.30 pg/ml in the animals with the prophylaxis; 56.11 ± 24.40 pg/ml in rats that had only VitC; and 43.93 ± 16.71 pg/ml in the control. The intoxicated rats with no prophylaxis expressed notably 43.5% lower levels of serum INF-ɣ compared to rats with prophylaxis ($P < 0.05$), and the animals with or without VitC ($P < 0.01$). Refer to **Fig.3** for more illustration of the cytokine levels in the serum of rats.

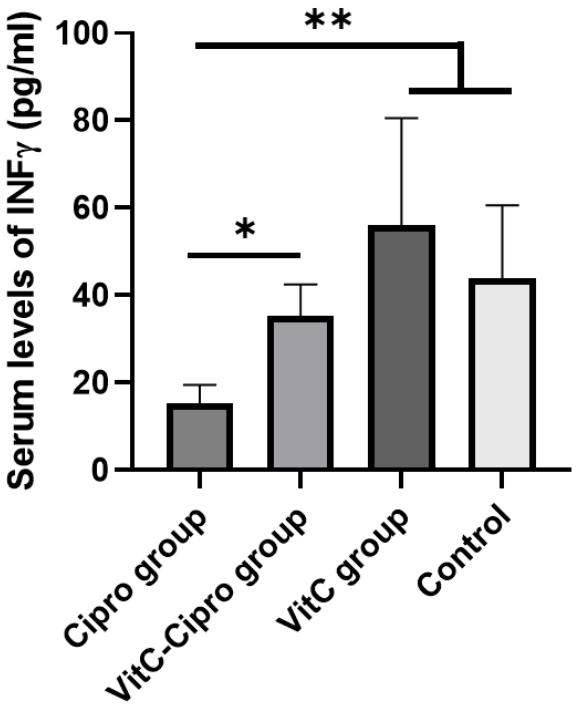

Fig.3 Mean concentrations (pg/ml) of INF-ɣ in serum of 4 groups of rats. From left to right: a group had 750 mg/kg cipro; a group had 200

mg/kg VitC then 1 hour later 750 mg/kg cipro; a group had VitC; and control had normal saline. All doses were orally given once daily for 5 days. Whiskers are representing standard errors

3.2. Histopathology Examination of Mesenteric Lymph Nodes

The histopathological changes are available in **Fig.4**, where Fig.4A represents tissue of mesenteric lymph nodes from cipro intoxicated rats without VitC prophylaxis. The lymph nodes had degenerative changes, mainly, moth-eaten appearance (circle), tingible body macrophages (TBMs) at the triangle point, localized hemorrhage, and reactive eosinophilia (arrow). TBMs are a subset of mononuclear large phagocytes that contain many phagocytized, apoptotic cells in various states of degradation. Fig.4B shows samples from VitC-cipro group, the paracortex tissue demonstrated a decreased quantity of lymphocytes with uneven distribution within the reticular tissue (star). Normal sections of mesenteric lymph nodes are in Fig.4C from VitC group and Fig.4D from control group. Lymphocytes are homogenously distributed throughout the lymph node tissue, no necrosis, no inflammation and no nodule formation.

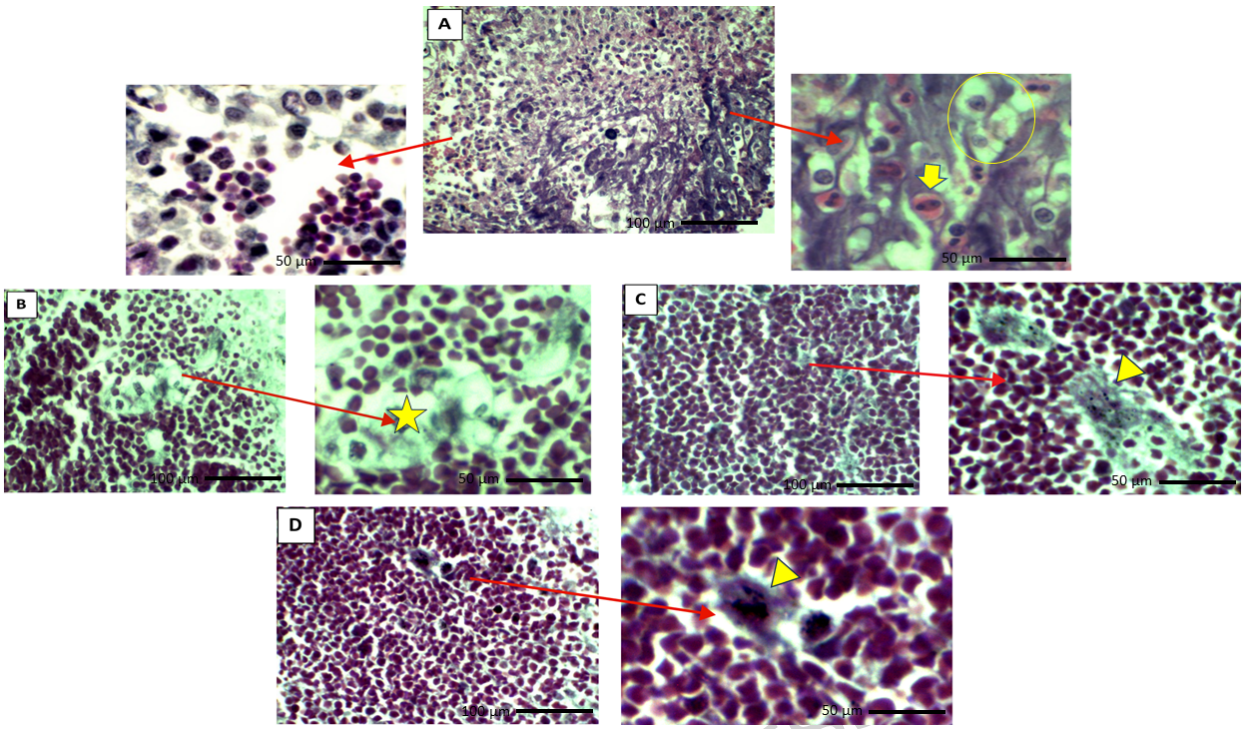

Fig.4 Histopathological sections of mesenteric lymph nodes from cipro group (A) at magnification of x100 with two enlarged fields to the left and to the right. Left (triangle), a higher magnification (x400) of a field from Image A, showing tingible body macrophages (TBM) with intracytoplasmic apoptotic bodies (arrows), which represent nuclear debris, surrounded by lymphocytes. Right (circle), a higher magnification of a field from Image A illustrating moth-eaten appearance, reactive eosinophilia with binuclearity, where cells clearly have two round nuclear lobes close together with joining filament (arrows). Image B shows a mesenteric lymph node section from cipro-VitC group, where the paracortex part demonstrated a decreased population of lymphocytes with uneven distribution throughout the reticular tissue. Image C as well as Image D show normal sections of

mesenteric lymph nodes with normal macrophages (arrow) from VitC group and the control group, respectively. Scale bar = 100 µm, 50 µm

Regarding quantitative analysis of the histological section, the average immune cells count was measured in 25 fields per 0.5 cm^2 area of lymph node tissue. Sections from the cipro intoxicated rats recorded an average of 233 ± 5.4 immune cells; sections from cipro intoxicated rats but had VitC prophylaxis recorded 106.3 ± 3.4 immune cells; sections from rats that had only VitC reported 192.5 ± 5.2 immune cells; and sections from control samples counted 190.5 ± 4.9 immune cells. The highest count of lymphocytes was recorded in intoxicated rats was due to the developed eosinophilia. Despite having VitC prophylaxis, notable decrease in the lymphocyte count was in the intoxicated rats relative to control. For statistical illustrations, **Fig.5** shows significant differences between Cipro group and all groups ($P < 0.001$). On the contrary, VitC prophylaxis reduced the numbers significantly compared to all groups ($P < 0.001$).

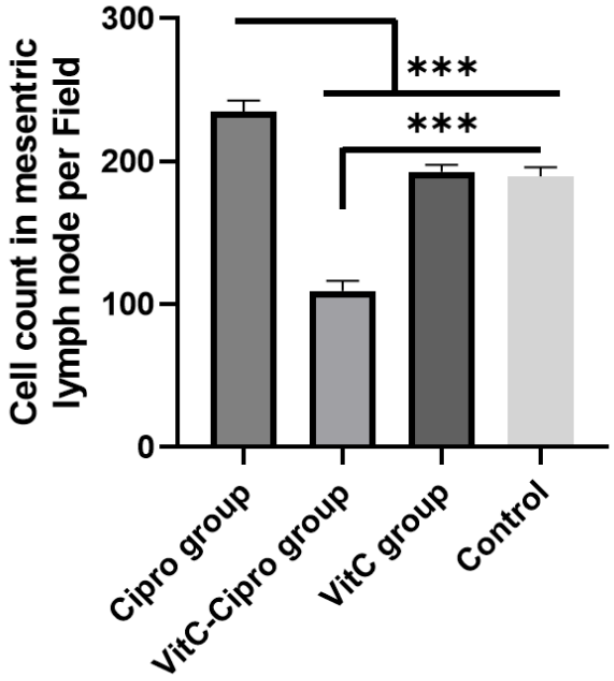

Fig.5 Average lymphocytes count per 25 fields of 4 groups of rats. From left to right: a group had 750 mg/kg cipro; a group had 200 mg/kg VitC then 1 hour later 750 mg/kg cipro; a group had VitC; and control had normal saline. All doses were orally given once daily for 5 days. Whiskers are representing one standard deviation

4. Discussion

This is the first report of immunotoxicity due to oral overdose exposure to the antibiotic cipro in rats and potential prophylactic action of VitC. The toxic effects were evaluated by measuring serum levels of IL-4, IL-10, and INF-ɣ to evaluate systemic impact on the immune system, and microscopic examination of mesenteric lymph nodes to assess local reaction in the

immune system. The selected dose of Cipro was based on a number of factors. First, Cipro has an oral bioavailability of approximately 70-80% [22], which significantly impacts effective dosing. In adults, typical dosages range from 250 mg to 750 mg taken twice daily [23], depending on the specific infection being treated. Second, a dose of 1000 mg/day is already on the higher end of rat dosing and within the guidelines to perform Repeated Dose Oral Toxicity (OECD Test Guideline 407) [24]. Third, research utilizing higher doses in controlled environments, such as animal models or clinical trials, aims to identify effects that may not be evident at standard dosages. These studies often incorporate safety monitoring to ensure their findings are applicable to clinical situations, with higher doses providing critical insights into potential risks.

IL-4 is a key anti-inflammatory cytokine that plays a critical role in the immune response to parasites and allergens [25]. In our findings, serum IL-4 levels were significantly lower in ciprofloxacin-intoxicated rats compared to controls, regardless of administered agents. This aligns with previous studies on albino mice, which indicated that treatment with Cipro at a dosage of 80 mg/100 g body weight led to a significant decrease in IL-4 production after 14 days [26]. In neonatal rats with E. coli sepsis, ciprofloxacin treatment also resulted in decreased serum IL-4 levels [27]. Overall, our results support the concept of Cipro-induced immunosuppression.

IL-10 serves as another important anti-inflammatory cytokine, crucial for maintaining immune homeostasis. It regulates various effector cells and mitigates allergic reactions and autoimmunity [28]. In our study, IL-10 serum levels did not show significant changes across all groups, regardless of treatment. This contrasts with previous reports where prophylactic Cipro treatment increased IL-10 concentrations in mesenteric lymph nodes of rats with colon cancer [29]. In a fish model, Cipro pre-exposure caused severe mucosal damage and inhibited IL-10 expression following subsequent infections [30]. The discrepancies in findings may stem from variations in experimental conditions and species studied.

IFN-ɣ, produced by T cells, and natural killer (NK) cells, plays a vital role in combating viral, bacterial, and protozoal infections [31]. Our results revealed significant decreases in serum IFN-ɣ levels in Cipro-intoxicated rats compared to other groups. Similar results have been documented, showing reduced IFN-ɣ production in splenocytes treated with Cipro in combination with chemotherapy [32]. Additionally, Cipro has been reported to dose-dependently decrease IFN-ɣ expression in lymphocytes from healthy individuals [33]. Our findings align with these studies indicating that ciprofloxacin significantly decreases IFN-ɣ levels. Furthermore, rats receiving VitC prophylaxis displayed normal IFN-ɣ levels, suggesting a protective effect of this antioxidant against antibiotic-induced immunotoxicity.

The histopathological examination of mesenteric lymph nodes corroborates the cytokine measurements. Lymph nodes from Cipro-intoxicated rats without VitC prophylaxis exhibited several degenerative changes, including localized hemorrhage, a moth-eaten appearance, and reactive eosinophilia. Reactive eosinophilia is characterized by binuclearity, defined by two round nuclear lobes connected by filaments [34]. Tingible body macrophages (TBMs) were also observed, indicating the presence of mononuclear phagocytes containing phagocytosed apoptotic cells at varying degradation stages. TBMs can actively phagocytose apoptotic lymphocytes, and the tingible bodies in their cytoplasm are referred to as apoptotic bodies [34]. In contrast, the reticular tissue within the paracortex of the VitC-Cipro group showed a decreased population of Immune cells. Control rats and those receiving only VitC displayed normal lymph node architecture, with a homogeneous distribution of macrophages and lymphocytes throughout the cortex and paracortex. These findings suggest a localized immune reaction following cipro overdose.

Eosinophilia can arise from numerous non-neoplastic causes, including allergic disorders, drug effects, and parasitic infections [34]. The observed eosinophilia in the mesenteric lymph nodes of Cipro-intoxicated rats, reflected in the elevated lymphocyte counts, underscores the drug's immunotoxic effects. Notably, the absence of eosinophilia in sections from intoxicated rats receiving VitC prophylaxis suggests a protective role for

VitC against Cipro-induced immunotoxicity, aligning with previous studies demonstrating the ability of VitC to mitigate antibiotic toxicity [35, 36].

This study has several limitations. First, the sample size, while consistent with similar toxicological studies, was not determined by a formal power calculation. Second, the study was conducted exclusively in male rats to control for hormonal variability; future studies should include female subjects to assess for sex-based differences. Third, the histopathological analysis was performed on lymph nodes from a subset of animals (n=2 per group); a larger sample size for histology would strengthen these findings. Fourth, while the high dose of Cipro was chosen to model overdose conditions, it may not directly reflect clinical exposure levels, and the findings should be interpreted within this experimental context. Finally, the protective mechanism of VitC, while likely linked to its antioxidant properties, was not mechanistically probed and warrants further investigation.

5. Conclusions

In summary, the current work sheds a new light on the systemic immunotoxic effect of cipro overdose by evaluating the serum concentrations of two anti-inflammatory cytokines (IL-4 and IL-10) and one proinflammatory cytokines (IFN-ɣ). The local immunotoxic effect of cipro overdose was evaluated by examining histological alterations in mesenteric lymph nodes. Both IL-4 and INF-ɣ were significantly decreased in the

serum of cipro intoxicated rats. IL-10 levels did not significantly change between all groups. Intoxicated animals with no prophylaxis expressed degenerative changes in the draining lymph nodes, mainly, moth-eaten appearance, TBMs and reactive eosinophilia. The revealed alterations in the selected interleukins, together with the changes in the mesenteric lymph node could support clinical assessment of cipro induced immunotoxicity. Furthermore, VitC prophylaxis ameliorated these immunotoxic effects, which encourages future investigations to adjust doses and administration protocols of this antioxidant as a prophylactic agent against antibiotics immunotoxicity.

Conflict of Interest Statement

The authors declare none.

Funding

This research was funded by the [Deanship of Research at Jordan University of Science and Technology] under grant [number 20200071].

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

All the listed authors participated in the manuscript. S.N. designed the study, participated in the experiments, analyzed the data and wrote the manuscript; H.A. carried animal experiments, laboratory tests, data collection, interpretation of results and manuscript drafting; K.A. participated in the data analysis, results interpretation and manuscript writing with critical reviewing. O.A., K.N. and N.A. participated equally in results interpretation and manuscript writing with critical reviewing.

References

1. Adikwu E& Brambaifa N. Ciprofloxacin cardiotoxicity and hepatotoxicity in humans and animals. *Pharmcology & Pharmacy*. **03**, 207-213; 10.4236/pp.2012.32028 (2012).

2. Lipman J, Allworth A& Wallis SC. Cerebrospinal fluid penetration of high doses of intravenous ciprofloxacin in meningitis. *Clinical Infectious Diseases*. **31**, 1131-1133; 10.1086/317462 (2000).

3. Du J, Liu Q, Pan Y, Xu S, Li H& Tang J. The Research Status, Potential hazards and toxicological mechanisms of fluoroquinolone antibiotics in the environment. *Antibiotics*. **12**, 1058; (2023).

4. Almutairi BAA, Almotawa AA, Almutairi MAM, Baabbad RS, Abdullah S AA, Alagedi HSK, Aljaizani EAH, Alahmar MA, Shabi FAA, Alharbi SA, Alaboudi ISA, Kaabi AA& Saleh A AA. Ciprofloxacin: An overview of uses, mechanism of action, and

adverse effects. *Journal of Ecohumanism*. **3**, 9328 – 9336; 10.62754/joe.v3i8.5546 (2024).

5. Kashyap A, Sreenivasan S, Rajan AK, Rashid M& Chhabra M. Ciprofloxacin-induced cutaneous adverse drug events: a systematic review of descriptive studies. *Journal of Basic and Clinical Physiology and Pharmacology*. **33**, 327-346; (2022).

6. Francis JK& Higgins E. Permanent peripheral neuropathy:A case report on a rare but serious debilitating side-effect of fluoroquinolone administration. *Journal of Investigative Medicine High Impact Case Reports*. **2**, 2324709614545225; 10.1177/2324709614545225 (2014).

7. Sharifzadeh S, Mohammadpour AH, Tavanaee A& Elyasi S. Antibacterial antibiotic-induced drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome: a literature review. *European Journal of Clinical Pharmacology*. **77**, 275-289; 10.1007/s00228-020-03005-9 (2021).

8. Richards GA, Brink AJ& Feldman C. Rational use of the fluoroquinolones. *South African Medical Journal*. **109**, 378-381; 10.7196/samj.2019.v109i6.14002 (2019).

9. Ibrahim NA, Buabeid MA, Elmorshedy KE& Arafa E-SA. Cell protective effects of vitamin C against oxidative stress induced by

ciprofloxacin on spermatogenesis: involvement of cellular apoptosis. *Frontiers in Cell and Developmental Biology*. **13**; 10.3389/fcell.2025.1489959 (2025).

10. Masadeh MM, Alzoubi KH, Al-azzam SI, Khabour OF& Al-buhairan AM. Ciprofloxacin-Induced Antibacterial Activity Is Atteneuated by Pretreatment with Antioxidant Agents. *Pathogens*. **5**, 28; (2016).

11. Weber B, Lackner I, Haffner-Luntzer M, Palmer A, Pressmar J, Scharffetter-Kochanek K, Knöll B, Schrezenemeier H, Relja B& Kalbitz M. Modeling trauma in rats: similarities to humans and potential pitfalls to consider. *Journal of Translational Medicine*. **17**, 305; 10.1186/s12967-019-2052-7 (2019).

12. Linster CL& Van Schaftingen E. Vitamin C. *The FEBS Journal*. **274**, 1-22; https://doi.org/10.1111/j.1742-4658.2006.05607.x (2007).

13. Odumosu ABI& Wilson CWM. Metabolic Availability of Vitamin C in the Guinea-pig. *Nature*. **242**, 519-521; 10.1038/242519a0 (1973).

14. West KL& Luz Fernandez M. Guinea pigs as models to study the hypocholesterolemic effects of drugs. *Cardiovascular Drug Reviews*. **22**, 55-70; 10.1111/j.1527-3466.2004.tb00131.x (2004).

15. Nusair SD& Ahmad MI. Toxicity of *Vipera palaestinae* venom and antagonistic effects of methanolic leaf extract of *Eryngium*

creticum lam. *Toxicon*. **166**, 1-8; 10.1016/j.toxicon.2019.05.006 (2019).

16. Nusair SD, Ananbeh M, Zayed A, Ahmad MI& Qinna NA. Postmortem sampling time effect on toxicity biomarkers in rats exposed to an acute lethal methomyl dose. *Toxicology Reports*. **9**, 1674-1680; 10.1016/j.toxrep.2022.08.010 (2022).

17. Sasi S, Toumi S, Awayn N, Saadawi W& Prastiya R. Ciprofloxacin Affect Sperm Quality and Induced Testicular and Hepatic Injury in Male Mice. *AlQalam Journal of Medical and Applied Sciences*. 477-483; (2024).

18. Olatunde B, Ojeka S& Dapper D. Protective Effects of Vitamin C, Vitamin E, and Levamisole on the Lead-induced Thrombocytopenia in Male Wistar Rats. *Archives of Current Research International*. **24**, 333-341; (2024).

19. Nusair SD, Joukhan AN, Rashaid AHB& Rababa'h AM. Methomyl induced effect on fortilin and S100A1 in serum and cardiac tissue: Potential biomarkers of toxicity. *Human & Experimental Toxicology*. **38**, 371-377; 10.1177%2F0960327118814153 (2019).

20. Nusair SD, Zainalabdeen EA, Alshogran OY& Alkaraki A. Evaluation of orellanine-induced toxicity from the mushroom

Cortinarius orellanus and the antagonistic effect of Petroselinum crispum. *Toxicon*. **214**, 1-7; 10.1016/j.toxicon.2022.04.018 (2022).

21. Nusair SD, Almasaleekh MJ, Abder-Rahman H& Alkhatatbeh M. Environmental exposure of humans to bromide in the Dead Sea area: Measurement of genotoxicy and apoptosis biomarkers. *Mutat Res Genet Toxicol Environ Mutagen*. **837**, 34-41; 10.1016/j.mrgentox.2018.09.006 (2019).

22. Sharma PC, Ankit J, Sandeep J, Rakesh P& and Yar MS. Ciprofloxacin: review on developments in synthetic, analytical, and medicinal aspects. *Journal of Enzyme Inhibition and Medicinal Chemistry*. **25**, 577-589; 10.3109/14756360903373350 (2010).

23. Lugg J, J. L, H. S& and Agarwal V. Determination of the concentration of ciprofloxacin in prostate tissue following administration of a single, 1000 mg, Extended-Release Dose. *Journal of Chemotherapy*. **20**, 213-218; 10.1179/joc.2008.20.2.213 (2008).

24. OECD. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing. 10.1787/9789264070684-en. (2008).

25. Keegan AD, Leonard WJ& Zhu J. Recent advances in understanding the role of IL-4 signaling. *Faculty Reviews*. **10**, 71; 10.12703/r/10-71 (2021).

26. Atallah AH, Masaoodi NNA& Obaid KH. Evaluation of the effect of ciprofloxacin on some cytokines in mice. *Journal of Pharmaceutical Negative Results*. **13**; (2022).

27. Loron G, Olivier P, See H, Le Saché N, Angulo L, Biran V, Brunelle N, Besson-Lescure B, Kitzis MD& Pansiot J. Ciprofloxacin prevents myelination delay in neonatal rats subjected to E. coli sepsis. *Annals of Neurology*. **69**, 341-351; (2011).

28. Boonpiyathad T, Satitsuksanoa P, Akdis M& Akdis CA, editors. Il-10 producing T and B cells in allergy. *Seminars in Immunology*; 2019: Elsevier.

29. Xue H, Field CJ, Sawyer MB, Dieleman LA& Baracos VE. Prophylactic ciprofloxacin treatment prevented high mortality, and modified systemic and intestinal immune function in tumour-bearing rats receiving dose-intensive CPT-11 chemotherapy. *British Journal of Cancer*. 100, 1581-1588; 10.1038/sj.bjc.6605051 (2009).

30. Wu XY, Xiong JB, Fei CJ, Dai T, Zhu TF, Zhao ZY, Pan J, Nie L& Chen J. Prior exposure to ciprofloxacin disrupts intestinal

homeostasis and predisposes ayu (Plecoglossus altivelis) to subsequent Pseudomonas plecoglossicida-induced infection. *Zoological Research* . **43**, 648-665; 10.24272/j.issn.2095-8137.2022.159 (2022).

31. Juanola O, Caparrós E& Francés R. Acute bacterial and viral infections. Sinusoidal Cells in Liver Diseases: Elsevier; 2024. p. 229-260.

32. Xue H, Sawyer M, Field C, Dieleman L& Baracos V. Prophylactic ciprofloxacin treatment prevents high mortality and modifies systemic and intestinal immune function in tumor-bearing rats receiving dose-intensive irinotecan chemotherapy. *Cancer Research*. **68**, LB-189-LB-189; (2008).

33. Williams AC, Galley HF, Watt AM& Webster NR. Differential effects of three antibiotics on T helper cell cytokine expression. *Journal of Antimicrobial Chemotherapy*. **56**, 502-506; (2005).

34. Morales-Camacho RM, Caballero-Velázquez T, Borrero JJ, Bernal R& Prats-Martín C. Hematological Neoplasms with Eosinophilia. *Cancers*. **16**, 337; (2024).

35. Raeeszadeh M, Karimfar B, Amiri AA& Akbari A. Protective Effect of Nano-Vitamin C on Infertility due to Oxidative Stress Induced by

Lead and Arsenic in Male Rats. *Journal of Chemistry*. 2021, 9589345; 10.1155/2021/9589345 (2021).

36. Raeeszadeh M, Mahtab R, Abolfazl A& and Khademi N. The comparison of the effect of *Origanum vulgare* L. extract and vitamin C on the gentamycin-induced nephrotoxicity in rats. *Drug and Chemical Toxicology*. 45, 2031-2038; 10.1080/01480545.2021.1895826 (2022).