Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2025, 38(4)

Evaluation of Interaction Ciprofloxacin- Ascorbic Acid against *Escherichia coli* Inducing Urinary Tract Infection in Rats

Tabark F. Ahmed [1*] and Nibras Z. Yahya[2]
[1,2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
*corresponding author

Received: 5 July 2023 Accepted: 10 September 2023 Published: 20 October 2025
doi.org/10.30526/38.4.3648

Abstract

Escherichia coli O157 urinary tract infections are quite widespread all over the world. Few clinical investigations have indicated that ascorbic acid supplementation shortens the duration and symptoms of urinary tract infections. The purpose of this study was to determine whether ascorbic acid was impacted by the antibiotic therapy indicated for urinary tract isolates of *E. coli O157*. Our study aimed to determine the efficacy of ciprofloxacin, ascorbic acid, or a combination of both in eliminating Escherichia coli O157 bacteria that had grown in the urethra of male Wistar albino rats. Following infection events, the animals exhibited significant increases in serum and urine biochemical markers linked to kidney damage. We infected the animals with Escherichia coli O157 and treated them with ciprofloxacin, ascorbic acid, and a combination of ciprofloxacin and ascorbic acid orally once daily for 7 days. Animals treated with the combination displayed a significant improvement and disappearance of clinical signs during the first days of treatment, as well as the return of serum urine creatinine and serum albumin concentrations to normal levels at the end of the treatment period. The histological analysis of the kidney section in the positive control group revealed multifocal tubules with necrotic findings, sloughed epithelium in dilated tubules, and widespread infiltration of different cell types. After three days of therapy, the combination group showed an intact histological structure, similar to the negative control group. However, it was determined that in vivo study against *Escherichia coli O157* in the treatment of urinary tract infection, the combination of ascorbic acid and ciprofloxacin was more potent and safer than ascorbic acid and ciprofloxacin separately.

Keywords: Ascorbic acid, *Escherichia coli O157,* Urinary tract infection, Ciprofloxacin

1.Introduction

Urinary Tract Infections (UTIs) are considered one of the most common infections and also a serious public health issue that are carried on by bacteria, but most commonly by Escherichia coli, Klebsiellapneumoniae, Proteusmirabilis, Enterococcus faecalis, and Staphylococcus saprophyticus., with 150 million people annually affected worldwide (1). Both Gram-negative and Gram-positive bacteria, as well as some fungi, are responsible for UTIs. In terms of the bacteria that cause uncomplicated UTIs, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B streptococcus (GBS), *Proteus*

mirabilis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida* spp. follow UPEC in prevalence (2). Most E. coli infections occur in the urinary tract, and E. coli infections account for more than 90% of all uncomplicated urinary tract infections (UTIs) (3). Multidrug-resistant (MDR) Escherichia coli urinary tract infection rates have sharply increased (4). *E. coli* helps with regular digestive functions. Even while the majority of E. coli do not cause disease, some of them do cause infections, such as urinary tract infections, as well as gastrointestinal and respiratory disorders (5). High rates of recurrence and rising antibiotic resistance among UPEC pose a serious threat to raising the cost of these UTIs. Commensal *E. coli* strains were generally sensitive to a wide range of antibiotics (6); however, because of the widespread and unrestricted usage of farm animals, antibiotic-resistant strains have grown to be a significant problem (7). Among various diseases caused by *E. coli*, urinary tract infections (UTIs) commonly occur (8). Due to its virulence genes, E. coli is considered a significant cause of diarrhea in adult dogs and puppies (9). A variety of virulence factors influence the pathogenicity of *Escherichia coli* isolates, the most prevalent cause of urinary tract infections (UTIs) (10). Broad-spectrum antibiotics like fluoroquinolones are very important for treating serious bacterial infections (11), especially hospital-acquired infections and other bacterial diseases that are likely to be resistant to other types of antibiotics. Ciprofloxacin is one of the few oral antibiotics to treat *P. aeruginosa* infections (12). Ascorbic acid is an antibacterial chemical that considerably reduces the adverse effects of reactive species and can change the antimicrobial activity of different medicines. Ascorbic acid is an important antibacterial, pro-oxidant, free radical scavenger, and antioxidant molecule that can change how different antibiotics kill microbes and greatly lessen the harmful effects of reactive species (13). It was concluded from his research that ascorbic acid plays a significant role as an antioxidant by enhancing the nervous system function of newborn rats whose mothers had been exposed to hydrogen peroxide in drinking water (14).

2. Materials and Methods

2.1. Experimental animal

The College of Veterinary Medicine at Baghdad University provided 25 male rats, 3 months old and weighing 200–250 g, for the current study. A typical animal facility housed the rats.

2.2. Experimental design

We divided twenty-five male rats into five groups, with five serving as controls and the remaining twenty rats infected. The infected group received intraurethral inoculations with *E. coli O157* bacteria by injecting 0.1 ml (2.6×10^6) CFU/ml of the bacterial suspension. The treatment begins 2 days after inducing infection. The first five rats were left untreated, and the 15 rats were treated as follows: (5 rats) treated with ciprofloxacin 14.2 mg/kg, (5 rats) treated with ascorbic acid 14.2 mg/kg, and finally (5 rats) treated with ASCORBIC/CIP 14.2 mg/kg. All animals were treated orally once daily for 7 days.

2.3. Clinical examination

2.3.1. Clinical Signs

For two days, we monitored all *E. coli*-infected rats in the various experimental groups for clinical symptoms such as urine color, unusually frequent urination, cloudy urine or bad-smelling pee, behavioral abnormalities, and activity levels. Throughout the experiment, we regularly monitored all animal groups.

2.3.2. Blood and urine samples

We collected blood and urine samples at zero and seven days to estimate the levels of urine creatinine and serum albumin. We followed the manufacturer's instructions to complete them.

2.4. Histopathological Examination

We removed the kidneys of each rat from the sacrificed animals and took samples for histopathological analysis. We preserved these samples in 10% neutral-buffered formalin, processed them according to standard procedures, wrapped them in paraffin, and then sliced and stained them with hematoxylin and eosin. (15).

2.5. Statistical Analysis:

To identify the impact of various factors on study parameters, the Statistical Analysis System (SAS) application was employed (2012). We used the least significant difference (LSD) test (ANOVA) to compare significant differences between means.

3. Results

3.1. Clinical signs

Before the induction of infection, all healthy animals exhibited normal feces and yellow urine. However, two days after infection, the animals began to show signs of anorexia, dehydration, and fever, and their urine turned dark yellow (16). From the first day post-infection, an increase in urination frequency was observed. Infected groups also showed changes in behavior and activity, along with cloudy and foul-smelling urine. Common clinical signs in all infected animals included fever, dehydration, crowding behavior, dullness, and difficulty or frequency in urination (**Figure 1**).

According to (17), 24 hours after *E. coli* infection, 5 out of 15 animals presented with frequent urination, dyspnea, emaciation, and diarrhea by the third day. At this point, the combination treatment group received medication at a dose of 14.2 mg/kg. Compared to the ciprofloxacin-only and ascorbic acid-only groups (treated at the same dosage), the combination group (B.W.) recovered faster and more completely, showing improved clinical signs. Full recovery was observed after seven days of treatment.

As reported in (18), rats with *E. coli*-induced urinary tract infections exhibited a progression of symptoms including fever, cloudy and bad-smelling urine, appetite loss, and lethargy within 24 to 48 hours of infection.

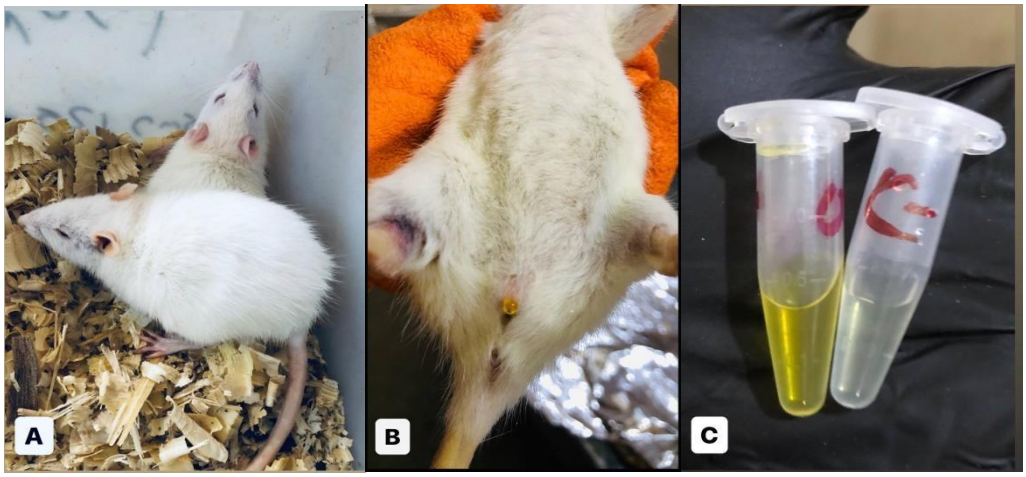

Figure 1. Acute UTI infected rats with *E. coli* O157 before treatment. **A-** Calming, dullness and rough hair, **B-** Cloudy urine, **C-** Compare between urine in control positive and control negative groups.

3.2. Serum creatinine concentration (mg/dl):

In **Figure 2**, serum creatinine values after inducing infection show a significant increase ($p \leq 0.05$) in all experimental groups (control positive group, ascorbic acid group, ciprofloxacin group, and combination group) when compared with the control negative group (-ve). After 7 days of treatment, it's worth mentioning there was no significant difference ($p \leq 0.05$) between the combination group and the control negative group, but there had been a significant improvement ($p \leq 0.05$) between the combination group and the ciprofloxacin group and a significant improvement ($p \leq 0.05$) between the combination group and the ascorbic acid group.

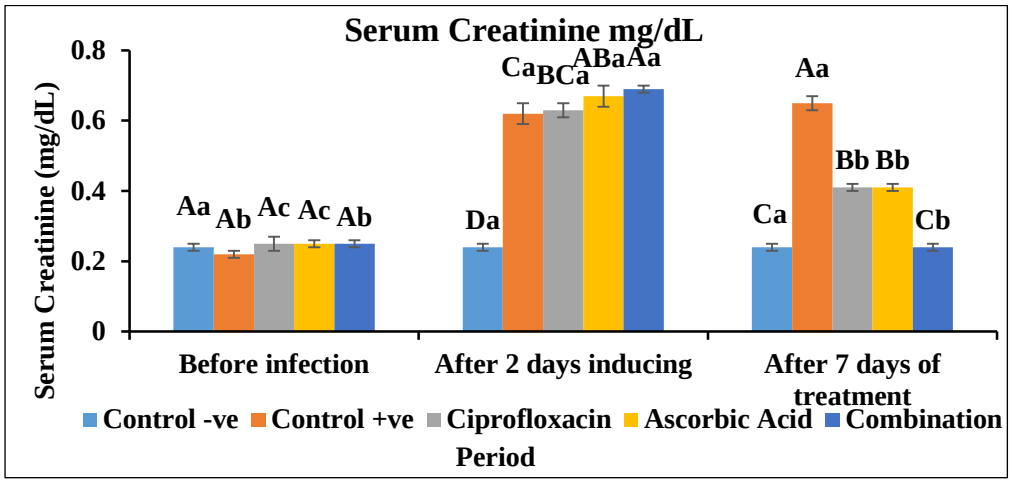

Figure 2. Serum Creatinine values of rats infected with *E. coli* and treated with Ascorbic acid and Ciprofloxacin each alone and in combination. A-C Significant differences at level $p \leq 0.05$ between groups. a-c Significant differences at level $p \leq 0.05$ within groups. Data as M±SE, LSD value=0.08

3.3.Serum albumin concentration

As shown in **Figure 3**, serum albumin levels after 2 days of induction were significantly higher in all experimental groups (control positive group, ascorbic acid group, ciprofloxacin group, and combination group) when compared with the control negative group.

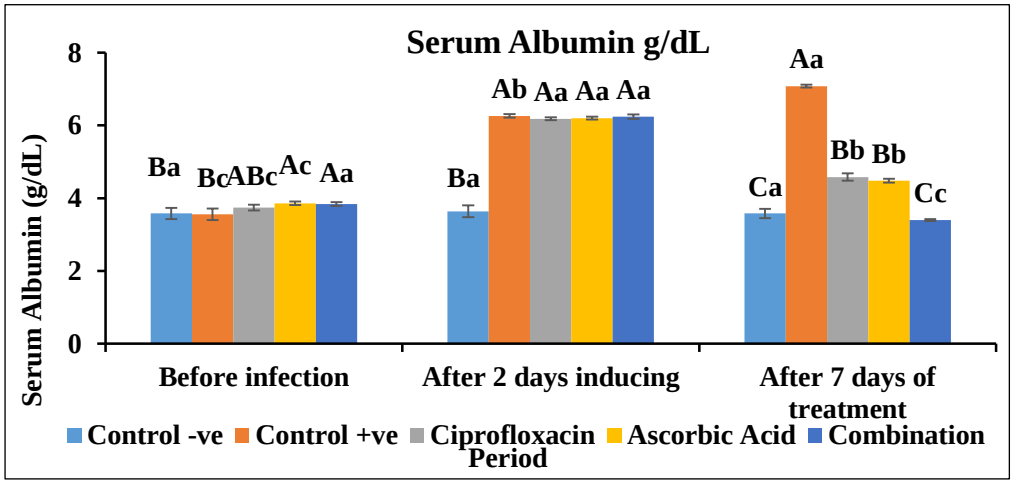

Figure 3. A-C Significant differences at level $p \leq 0.05$ between groups. a-c Significant differences at level $p \leq 0.05$ within groups. Data as M±SE**,** LSD value=0.25.

After 7 days of treatment, all treated groups (ascorbic acid group, ciprofloxacin group, and combination group) show significant decreases ($P \leq 0.05$) when compared with the control negative group, except the combination group shows no significant differences ($P \leq 0.05$) when compared with the control negative group, which is superior to the ciprofloxacin group and ascorbic acid group.

3.4.Urine creatinine concentration

As shown in Figure 4, urine creatinine levels after 2 days of induction were significantly higher in all experimental groups (control positive group, ascorbic acid group, ciprofloxacin group, and combination group) when compared with the control negative group. After 7 days of treatment, all treated groups (ascorbic acid group, ciprofloxacin group, and combination group) show a significant decrease (P≤0.05) when compared with the control negative group, except the combination group shows a significant decrease (P≤0.05) when compared with the ciprofloxacin group and ascorbic acid group and shows no significant difference when compared with the control negative group (P≤0.05).

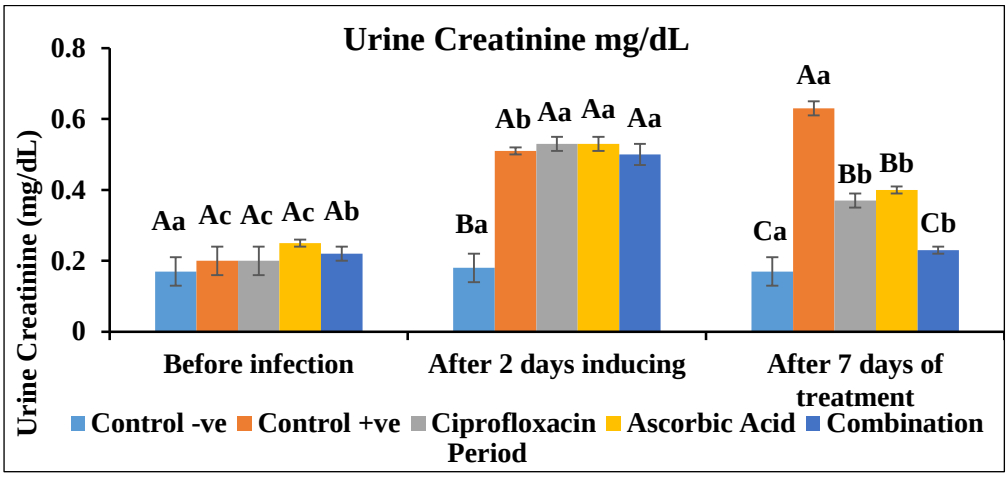

Figure 4. Urine creatinine values of rats infected with *E. coli O157* and treated with Ascorbic acid and Ciprofloxacin each alone and in combination**.** A-C Significant differences at level p≤0.05 between groups. a-c Significant differences at level p≤0.05 within groups. Data as M±SE, LSD value=0.08**.**

In the final stages of renal failure, elevations in serum creatinine and albumin were observed both are metabolic waste products filtered by the kidneys' glomeruli and commonly used as renal function markers (19, 20). Creatinine, a byproduct of muscle metabolism, is primarily eliminated by the kidneys via glomerular filtration. Blood creatinine tests were used as a reliable indicator of kidney function in this study (21).

The current study found that intraurethral catheterization with *E. coli* in rats led to cellular damage in the kidneys and urethra. The *E. coli* strain used possessed colonization factors that facilitated bacterial attachment to specific receptors in the kidney and urethral tissues. The bacteria produced Shiga toxin, an endotoxin that caused hemorrhaging in the kidney, urethra, and bladder lumen, leading to ulceration and further dissemination to critical organs.

Infected rats exhibited significantly increased serum creatinine and albumin levels. In contrast, rats treated with a combination of ciprofloxacin and ascorbic acid (once daily for 7 days) showed reduced serum creatinine, albumin, and urinary creatinine concentrations, indicating enhanced bacterial clearance. The combination therapy exhibited greater bactericidal activity against the *E. coli* O157 isolate than either drug used individually.

3.5.Histopathological changes in the kidneys

The histological examination of kidney tissues from the **negative control group** showed normal structural features, including intact glomeruli (Gr) and normal convoluted tubules (CT), with no pathological alterations observed (**Figure 5**).

In contrast, the positive control group exhibited notable histopathological changes, including multifocal tubular necrosis, sloughed epithelial cells within dilated tubules, and diffuse infiltration of inflammatory cells. The kidney tissue displayed varying degrees of inflammation (35) (**Figure 6**).

The ciprofloxacin-treated group demonstrated moderate swelling of the tubular epithelial lining, interstitial infiltration mainly composed of macrophages and lymphocytes, and mild cystic dilation in some renal tubules (**Figure 7**).

The group treated with ascorbic acid showed mild to moderate perivascular infiltration of mononuclear cells (MNCs), predominantly macrophages, along with cystic dilation of some tubules (**Figure 8**).

However, the combination treatment group (ciprofloxacin+ascorbic acid) revealed no significant pathological alterations in the glomerular structures. Only mild cellular swelling was observed in the renal tubules, indicating minimal tissue damage (**Figure 9**).

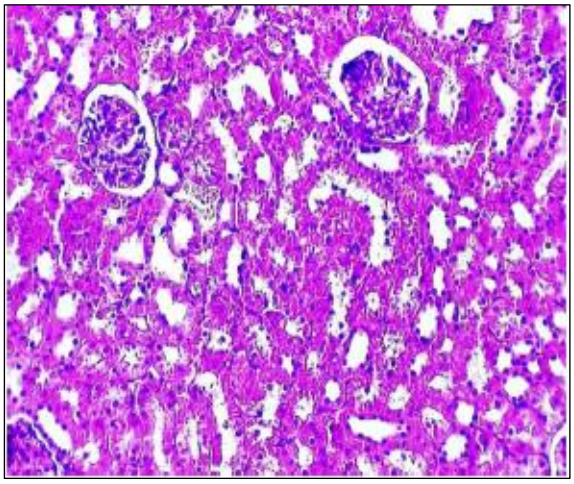

Figure 5. Kidney section of control group showed normal kidney structure (Glomerulus, Convoluted Tubules) (H & E stain 10X).

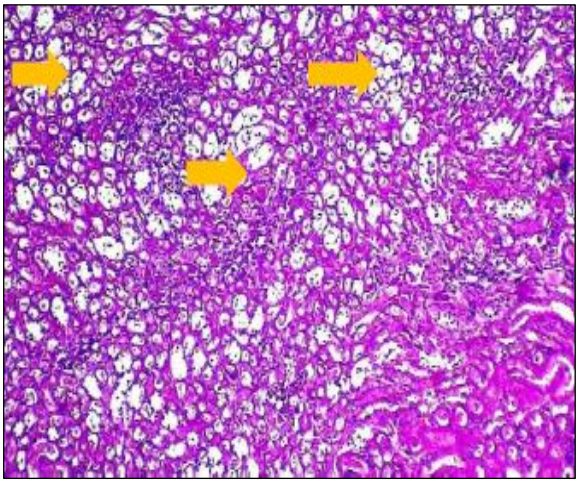

Figure 6. Kidney section of rat of control positive group showed multifocal tubular necrosis ➡ (H & E stain 10X).

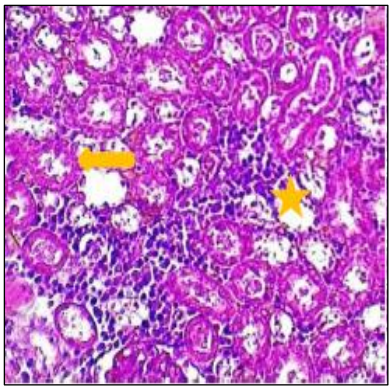

Figure 7. Kidney section in rat of treated ciprofloxacin group showed focal MNCs infiltration ★ with mild cystic dilation of some tubules ⬅ (H and E stain 10X).

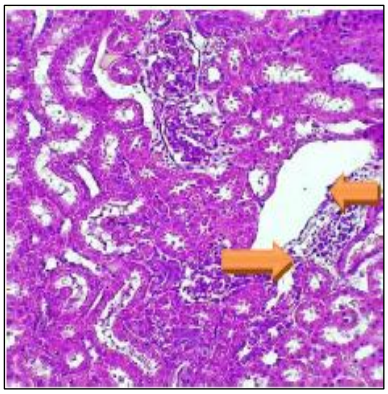

Figure 8. Kidney section of rat of treated ascorbic acid group showed moderate perivascular MNCs infiltration ⬅ (H and E stain 10X).

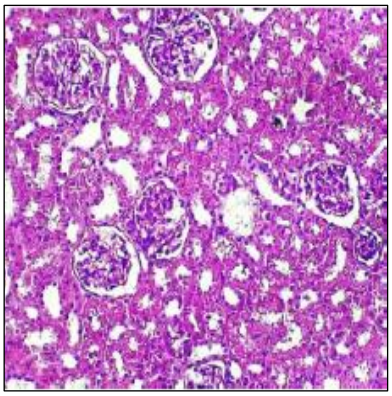

Figure 9. Kidney section of rat of combination (CIP/ASCORBIC) group showed no obvious pathological alteration in glomerular tissue except slight cellular swelling of renal tubules H and E (10 X).

4. Discussion

The observed clinical signs—such as fever, dark or cloudy urine, loss of appetite, and behavioral changes—are consistent with previous findings related to *E. coli*-induced urinary tract infections. The early onset of symptoms within 24–48 hours confirms the rapid progression of the infection in rodent models. Increased urination and the presence of foul-smelling urine suggest a strong inflammatory response in the urinary tract.

The therapeutic response noted in the combination treatment group (B.W.) indicates a synergistic effect when ciprofloxacin is combined with ascorbic acid. Faster and more

complete recovery compared to monotherapy groups suggests that this combination may enhance bacterial clearance or mitigate oxidative stress induced by infection.

These findings align with earlier studies highlighting the limitations of monotherapy in severe infections and support the potential benefit of adjunctive therapies in managing UTIs caused by *E. coli.*

The renal damage observed in this study is consistent with previous reports. According to (22), the Shiga toxin produced by *E. coli* can cause extensive hemorrhaging and cellular damage in renal tissues, which may lead to systemic toxemia and potentially fatal outcomes. These findings align with (23), who reported elevated creatinine levels and severe kidney damage in rabbits infected with pathogenic *E. coli*. Other studies have similarly linked increased creatinine levels to kidney responses to microbial infections and associated toxins (24-26).

The elevated serum albumin levels in this study mirror findings by (27), who reported similar increases in camels with UTIs, likely due to inflammatory responses and altered renal filtration. Furthermore, high creatinine clearance rates, possibly due to increased renal blood flow, can enhance the renal excretion of certain drugs (28), including hepatically cleared antibacterials.

Our results highlight the superior efficacy of the combination therapy of ciprofloxacin and ascorbic acid. The combination not only achieved complete bacterial eradication but also normalized renal function markers. The enhanced bactericidal effect may be attributed to ascorbic acid's ability to generate reactive oxygen species (ROS), which are known to disrupt bacterial growth through oxidative stress (29). As shown in previous studies (30, 31), ascorbic acid can inhibit pathogens like *Mycobacterium tuberculosis* by stimulating ROS production via Fenton's reaction (32).

In addition to its oxidative mechanism, ascorbic acid may reduce bacterial colonization through urine acidification, creating an inhospitable environment for uropathogens (33). Furthermore, it may act synergistically with antibiotics by enhancing their activity through ROS generation, as noted in (34).

The histopathological findings of this study provide further evidence of the protective effect of the combination therapy (ciprofloxacin and ascorbic acid) on renal tissues in rats with *E. coli*-induced urinary tract infections.

The normal histological architecture observed in the negative control group confirms the baseline integrity of renal tissues in healthy animals. In contrast, the positive control group exhibited extensive pathological damage, including tubular necrosis, sloughed epithelium, and significant inflammatory cell infiltration, which are hallmark signs of severe bacterial infection and associated immune response. These findings are consistent with previous studies that reported acute tubular injury and inflammation as consequences of *E. coli* infection in renal tissues (35).

Treatment with ciprofloxacin alone resulted in moderate improvements in tissue morphology, though some inflammatory infiltration and epithelial swelling persisted. This suggests that while ciprofloxacin exhibits effective antibacterial activity, it may not completely prevent infection-induced tissue damage.

Similarly, ascorbic acid alone reduced the severity of inflammation and tubular damage, likely due to its antioxidant and immunomodulatory properties. The presence of perivascular mononuclear cell infiltration indicates a mild ongoing immune response, but less severe than in the untreated infected group.

The most notable outcome was observed in the group treated with the combination of ciprofloxacin and ascorbic acid. Minimal pathological changes, limited to mild tubular cell swelling, were observed. The absence of glomerular damage and reduced inflammatory response suggest that the combination therapy not only enhanced bacterial clearance but also mitigated oxidative stress and protected kidney tissue from severe inflammatory injury.
These findings support the hypothesis that ascorbic acid enhances the therapeutic efficacy of ciprofloxacin, likely through its antioxidant mechanisms and ability to promote host defense. The reduced tissue damage observed in the combination group highlights the potential benefit of adjunctive antioxidant therapy in managing UTIs and protecting renal tissue.

5. Conclusion

This study showed that the combination of ciprofloxacin with ascorbic acid has a highly antibacterial activity against *E. coli-induced* UTI in a rat model. When mixed with antibiotics, ascorbic acid has high synergistic activity, increasing the potency of these antibiotics.

Acknowledgment

No thanks

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this research.

Funding

This research received no external funding.

References

1. Eckert L, Mattia L, Patel S, Okumura R, Reynolds P, Stuiver I. Reducing the risk of indwelling catheter-associated urinary tract infection in female patients by implementing an alternative female external urinary collection device: a quality improvement project. J Wound Ostomy Continence Nurs. 2020;47(1):50-53. https://doi.org/10.1097/WON.0000000000000601
2. Sahu R, Sahoo RK, Prusty SK, Sahu PK. Urinary Tract Infection and its management. Syst Rev Pharm. 2019;10(1):42-48. https://doi.org/10.5530/srp.2019.1.7.
3. Madappa T (2014) Escherichia coli infections. http://tinyurl.com/qfxuocb. (accessed 29 January 2015).
4. Mohammed SA, Ibrahim OMS. Pharmacodynamics analysis of fosfomycin against multi-drugs resistant E. coli O157:H7 isolated from urinary tract infection. Biochem Cell. Arch. 2022; 22(1): 1785-1791. https://connectjournals.com/file_full_text/3566001H_1785-1791.pdf.
5. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, Choroszy-Krol I. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathog. 2019;11:1-16. https://doi.org/10.1186/s13099-019-0290-0.
6. Rubab M, Oh DH. Virulence characteristics and antibiotic resistance profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources. Antibiotics. 2020;9(9):587. https://doi.org/10.3390/antibiotics9090587.
7. Abbas-Al-Khafaji ZK, Aubais-aljelehawy QH. Evaluation of antibiotic resistance and prevalence of multi-antibiotic resistant genes among *Acinetobacter baumannii* strains isolated from patients admitted to Al-Yarmouk Hospital. Cell Mol Biomed Rep. 2021;1(2):60-68. https://doi.org/10.55705/cmbr.2021.142761.1015.

8. Mraidi DA, Lafta IJ. RAPD-PCR and phylogenetic analysis of *E. coli* isolated from human and cattle urinary tract infections. Iraqi J Vet Sci. 2021;35:59-66. https://integration-badge.dimensions.ai/details/id/pub.1144305507.
9. Abdulrazzaq KM, Owain MS, Majeed HM, Al-Hyani O. Molecular detection of rfb0157, shiga toxins and hemolysin genes for *Escherichia coli* O157:H7 from canine feces in Tikrit and Mosul cities, Iraq. Iraqi J Vet Sci. 2021;35(2):325-329. http://www.doi.org/10.33899/ijvs.2020.126831.1392.
10. Ahmed NA, Mahmood SS, Abbas AH. A comparative study of some virulence factors and phylogenetic characterization of *Escherichia coli* isolates causing urinary tract infection and the commensal gut. Iraqi J Agric Sci. 2019;50(3):1193-1198. https://doi.org/10.36103/ijas.v50i4.763.
11. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Torres A. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44(Suppl 2):S27-72. https://doi.org/10.1086/511159.
12. Rehman A, Patrick WM, Lamont IL. Mechanisms of ciprofloxacin resistance in Pseudomonas aeruginosa: new approaches to an old problem. J Med Microbiol. 2019;68(1):1-10. https://doi.org/10.1099/jmm.0.000873.
13. Kwiecińska-Piróg J, Skowron K, Bogiel T, Białucha A, Przekwas J, Gospodarek-Komkowska E. Vitamin C in the presence of sub-inhibitory concentration of aminoglycosides and fluoroquinolones alters Proteus mirabilis biofilm inhibitory rate. Antibiotics. 2019;8(3):116. https://doi.org/10.3390/antibiotics8030116.
14. Jasim AY, Rasheed SA. Effect of vitamin C treatment on some central nervous system functions in young rats whose mothers treated with hydrogen peroxide during the lactation period. Iraqi J Vet Sci. 2021;35(4):713-717. https://doi.org/10.33899/ijvs.2021.127894.1544.
15. Suvarna KS, Layton C, Bancroft JD, editors. Bancroft's theory and practice of histological techniques e-Book. Elsevier Health Sciences; 2018.
16. Al-Iraqi OM. Urinary tract bacterial infection of local Iraqi buffaloes (*Bubalus bubalis*) in Mosul city. Iraqi J Vet Med. 2016;40(2):124-30. https://doi.org/10.30539/iraqijvm.v40i2.123.
17. Al-Taii DHF, Yousif AA. Effects of *E. coli* O157:H7 experimental infections on rabbits. Iraqi J Vet Med. 2019;43(1):34-42. https://doi.org/10.30539/iraqijvm.v43i1.468.
18. Woodford HJ, George J. Diagnosis and management of urinary infections in older people. Clin Med (Lond). 2011;11(1):80-83. https://doi.org/10.7861/clinmedicine.11-1-80.
19. Rodríguez-Cubillo B, Carnero-Alcázar M, Cobiella-Carnicer J, Rodríguez-Moreno A, Alswies A, Velo-Plaza M, Maroto-Castellanos L. Impact of postoperative acute kidney failure in long-term survival after heart valve surgery. Interact Cardiovasc Thorac Surg. 2019;29(1):35-42. https://doi.org/10.1093/icvts/ivz035.
20. Gai Z, Wang T, Visentin M, Kullak-Ublick GA, Fu X, Wang Z. Lipid accumulation and chronic kidney disease. Nutrients. 2019;11(4):722. https://doi.org/10.3390/nu11040722.
21. Onuigbo MAC, Agbasi N. Diabetic nephropathy and CKD-analysis of individual patient serum creatinine trajectories: a forgotten diagnostic methodology for diabetic CKD prognostication and prediction. J Clin Med. 2015;4(7):1348-68. https://doi.org/10.3390/jcm4071348.
22. Narins B. World of Microbiology and Immunology. Vol. 2. Thomson Gale; 2003. p. 381.
23. AL_Okhedi MJI, Najim TM, Al-Shammari BFM, Hasan MS, Jead MR. Effects of E. coli infection on kidney function tests in experimentally inoculated rats. Med Leg Update. 2020;20(4):41354. https://doi.org/10.37506/mlu.v20i4.2012.
24. Pai CH, Kelly JK, Meyers GL. Experimental infection of infant rabbits with verotoxin-producing *Escherichia coli*. Infect Immun. 1986;51(1):16-23.
25. Tzipori S, Karch H, Wachsmuth KI, Robins-Browne RM, O'Brien AD, Lior H, Levine MM. Role of a 60-megadalton plasmid and Shiga-like toxins in the pathogenesis of infection caused by enterohemorrhagic *Escherichia coli* O157:H7 in gnotobiotic piglets. Infect Immun. 1987;55(12):3117-25. https://doi.org/10.1128/iai.51.1.16-23.1986.

26. Kaper JB, O'Brien AD. *Escherichia coli O157:H7 and other Shiga toxin-producing E. coli strains.* ASM Press; 1998.
27. El-Deeb WM, Buczinski S. The diagnostic and prognostic importance of oxidative stress biomarkers and acute phase proteins in urinary tract infection (UTI) in camels. PeerJ. 2015;3:e1363. https://doi.org/10.7717/peerj.1363.
28. Roberts JA, Lipman J. Antibacterial dosing in intensive care: pharmacokinetics, degree of disease and pharmacodynamics of sepsis. Clin Pharmacokinet. 2006;45:755-73. https://doi.org/10.2165/00003088-200645080-00001.
29. Chen Y, Luo G, Yuan J, Wang Y, Yang X, Wang X, Zhong N. Vitamin C mitigates oxidative stress and tumor necrosis factor-alpha in severe community-acquired pneumonia and LPS-induced macrophages. Mediators Inflamm. 2014; 2014: 426740. https://doi.org/10.1155/2014/426740.
30. Vilchèze C, Hartman T, Weinrick B, Jacobs WR Jr. *Mycobacterium tuberculosis* is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. Nat Commun. 2013;4:1881. https://doi.org/10.1038/ncomms2898.
31. Yuan YG, Peng QL, Gurunathan S. Effects of silver nanoparticles on multiple drug-resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from mastitis-infected goats: an alternative approach for antimicrobial therapy. Int J Mol Sci. 2017;18(3):569. https://doi.org/10.3390/ijms18030569.
32. Sikri K, Duggal P, Kumar C, Batra SD, Vashist A, Bhaskar A, Tyagi JS. Multifaceted remodeling by vitamin C boosts sensitivity of *Mycobacterium tuberculosis* subpopulations to combination treatment by anti-tubercular drugs. Redox Biol. 2018;15:452-66. https://doi.org/10.1016/j.redox.2017.12.020.
33. Hassuna NA, Rabie EM, Mahd WKM, Refaie MM, Yousef RKM, Abdelraheem WM. Antibacterial effect of vitamin C against uropathogenic *E. coli in vitro* and *in vivo*. BMC Microbiol. 2023;23(1):1-12. https://doi.org/10.1186/s12866-023-02856-3.
34. Vickers NJ. Animal communication: when I'm calling you, will you answer too? Curr Biol. 2017;27(14):R713-5. https://doi.org/10.1016/j.cub.2017.05.064.
35. Ibrahim ZI and Alwaan MJ. Experimental infection of *Klebsiella pneumoniae* in urinary tract of rats and guinea pigs. Iraqi J Vet Med. 2008;32(2):68-79. https://doi.org/10.30539/iraqijvm.v32i2.740.