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# Ameliorative Effects of Vitamin E against Ceftriaxone-induced Adverse Effects in Broilers Challenged with *E. coli*

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## Abstract

Ceftriaxone is a broad spectrum antimicrobial that commonly used in chicken farms for the purpose of the control of avian bacterial diseases. However, the use of ceftriaxone can be associated with some adverse effects. This study examined vitamin E's protective properties against ceftriaxone-induced histopathological, inflammatory, and oxidative damage in broiler chickens challenged with *E. coli*. When broiler chicks are exposed to *E. coli* O78, colibacillosis was developed. The sickened birds displayed a variety of general clinical symptoms. Ceftriaxone was used as a treatment and could lower morbidity and fatality rates to 10% and 4%, respectively. The use of ceftriaxone was associated with the occurrence of some histopathological lesions. In addition, either *E. coli* or ceftriaxone could cause alterations in erythrogram, antioxidant status, and induction of inflammatory markers. Interestingly, the use of vitamin E with ceftriaxone could markedly improve the histopathological alterations and upregulate the antioxidant status and reduce the release of the inflammatory markers. In conclusion, the present study's findings showed that ceftriaxone is a powerful antibacterial drug, especially when used to treat *E. coli* in broilers. Vitamin E is strongly advised for usage in broilers to minimize ceftriaxone-induced adverse effects.

KEYWORDS
*E. coli*, Ceftriaxone, Vitamin E, Broiler chicken

## INTRODUCTION

In the past few decades, the production of chicken products has increased more quickly in the developing countries than the production of any other significant food. One way to meet the rising need for animal protein is through the increase in the poultry production. Chicken flesh is popular right now as a cheap protein source with low cholesterol levels (Razieh *et al*., 2015; Darwish *et al*., 2018).

The most significant agent causing secondary bacterial infection in chickens is *Escherichia coli* (*E. coli*), which may also be a major pathogen for different avian species such as chicken, turkeys, pigeon, and ducks (Saif, 2008; Darwish *et al*., 2015).

Antibiotics were administered to chicken through their feed and water in therapeutic doses to manage and prevent poultry infections, including colibacillosis (Bower and Daeschel, 1999). Moreover, antimicrobials are used as feed additives for the purpose of growth promotion (Alsayeqh *et al*., 2021).

Large-scale use of veterinary medication may cause antimicrobial residues to accumulate in an animal's muscles and organs. The effects of consuming these residues in animal products on consumers include the emergence of microorganisms resistant to antibiotics, the development of allergies, the alteration of the microbiota, diseases brought on by these factors, the failure of pharmacological therapy, reproductive issues, and hypersensitivity reactions (Darwish *et al*., 2013; Andrew and Oluwakamisi, 2019).

All antibiotics that have a beta-lactam ring in their molecular structures fall into the broad-spectrum antibiotic category known as beta-lactams. Penicillin and cephalosporins are examples of this (Holten and Onusko, 2000). Due to their broad-spectrum activity and safety, cephalosporins are frequently utilized in veterinary medicine. They are used as preventative measures during abdominal surgery as well as for the treatment of bacterial infections of the skin, urinary and vaginal tracts, respiratory tract, bones and joints, and soft tissues of the skin (Greene and Watson, 2001). Ceftriaxone is one of these antibiotics, and it is frequently utilized in clinical practice due to its excellent antibacterial efficacy. Ceftriaxone is a member of the 3rd generation of cephalosporin antibiotics and is used in both human and veterinary medicine (Cho *et al*., 2004). A broad-spectrum antibiotic for intramuscular or intravenous injection is ceftriaxone. It has a broad spectrum of activity, a high antibacterial efficacy, and a minimal potential for toxicity (Lee *et al*., 2009).

Chemicals called antioxidants stop free radicals from damaging tissue by neutralizing them. Antioxidants are chemicals that prevent or postpone oxidation of the substrate; however, they are extremely rare (Schieber and Chande, 2014). Antioxidants are currently used as dietary supplements in order to preserve health and prevent diseases like cancer and coronary heart disease


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because nutrition is the most significant source of antioxidants (Morikawa *et al*., 2000).

Vitamin E is a lipid-soluble, chain-breaking antioxidant that plays a significant protective function against oxidative stress and prevents the development of lipid peroxides in biological membranes. It is also helpful in reducing the harmful effects of medications and other chemicals (Atessahin *et al*., 2005). Through its antioxidant action that breaks antioxidant chains, vitamin E defends the integrity of cell membranes against lipid peroxidation (Brigelius, 2009; Darwish *et al*., 2020).

This study aimed to investigate the ameliorative effects of vitamin E against ceftriaxone-induced histopathological, inflammatory and oxidative damage in the *E. coli* challenged broiler chicken.

## MATERIALS AND METHODS

All animal testing was done in accordance with Zagazig University regulations. The ZU-IACUC/2/F/52/2022 ethical approval number was given to this project.

*Ceftriaxone*

SmithKline Beecham for Novartis Pharma Company, Egypt, provides ceftriaxone (Ceftriaxone) in dosages of 250 mg, 500 mg, and 1000 mg of ceftriaxone sodium for intramuscular (IM) or intravenous (IV) injection. The recommended dosage is 50 mg/kg bwt over the course of five consecutive days (Pardeep *et al*., 2011).

*Bacteria*

The Animal Health Research Institute, EL-Dokki, Cairo, Egypt, kindly contributed *E. coli* O78 strains.

*Experimental groups*

Cobb broiler chicks of 200 day-old were purchased from Egypt's El-Dakahlea Poultry Company were used for this experiment. Clean cages, a balanced diet free of drugs, and unfettered access to food and water were provided for broilers. Gp1: Healthy broilers, held as a control group, was one of four equal groups of broilers (n=50/group). Gp2: This group was exposed to *E. coli* O78 and was not treated. Gp3: After two days of an experimental infection, Gp3 was given ceftriaxone at a dose of 50 mg/kg bwt for five consecutive days. Gp4 was exposed to *E. coli* O78, 3 days post infection chicks treated with ceftriaxone (50 mg /kg bwt IM) and vitamin E (100 mg /kg bwt in drinking water, El-Obour Company) for 5 successive days (Hamza and El-Shennawy, 2009). For those birds exposed to *E. coli*, clinical symptoms, morbidity, and mortality rates were also noted.

*Samples*

Blood samples were collected in either EDTA-containing tube for hematological studies according to Jain (2000), or without anticoagulants for estimation of the antioxidant enzymes. Antioxidant enzymes: Superoxide dismutase (SOD), glutathione peroxidase (Gpx), and malondialdehyde (MDA) were additionally evaluated according to the methods reported before (Draperi and Hadly, 1990). Tissue specimens were collected for the purpose of histopathology. Liver samples were also collected for RNA extraction to examine changes in the expression of IL10, and TNF-α.

*Histopathological examination*

Collected tissue samples from liver, kidney, spleen, and lung were fixed in 10% buffer formalin solution, processed with paraffin embedding technique and stained with hematoxylin and eosin then examined microscopically (Survarna *et al*., 2013).

*RNA extraction and analysis using qReal-Time PCR*

Total RNA was isolated from the liver samples in accordance with the manufacturer's instructions using the QIAamp RNeasy Mini kit from Qiagen, GmbH, Germany. To examine the expression of IL10, TNF-, and β-actin as a housekeeping gene, a 25 µl reaction including 10 µl of the 2x HERA SYBR® Green RT-qPCR Master Mix (Willowfort, UK), 1 µl of RT Enzyme Mix (20X), 5 µl of DDW, and 3 µl of RNA template was employed. Germany's Metabion contributed the primers (Strong *et al*., 2015). The initial reaction was conducted using a real-time PCR system. CT readings and amplification curves were computed using Step One Software. The "Ct" approach was used to compare the CT of each sample to that of the positive control group (Yuan *et al*., 2006).

*Statistical analysis*

The gathered data were analyzed using one-way analysis of variance (ANOVA) and the Tukey's HSD test using the computerized SPSS program version 16 (Tambane and Dunlop, 2000).

## RESULTS AND DISCUSSION

When broiler chicks are exposed to *E. coli* O78, colibacillosis develop. The sickened birds displayed a variety of general clinical symptoms, such as loss of appetite, diarrhea, dehydration, and weakness. The dead birds' postmortem examination revealed pericarditis, perihepatitis, and air sacculitis as well as liver enlargements with fibrinous perihepatitis. The mortality rate was 20%, while the morbidity rate was 60%. Prior reports of clinical symptoms and postmortem abnormalities were similar (Lutful Kabir, 2010). In the second day following the onset of symptoms,

Table 1. Ameliorative effects of vitamin E. against ceftriaxone-induced alterations in Hb, RBCs, and PCV in broilers challenged with *E. coli*

| | PCV (%) | | | | RBCs (x10$^6$/µl) | | | | Hb (g/dl) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1$^{st}$ Day | | 7$^{th}$ Day | | 1$^{st}$ Day | | 7$^{th}$ Day | | 1$^{st}$ Day | | 7$^{th}$ Day | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Control | 22.99a | 0.22 | 23.78a | 0.15 | 2.22a | 0.03 | 2.49a | 0.04 | 7.69a | 0.02 | 7.82a | 0.02 |
| *E. coli* | 20.64a | 0.16 | 19.41b | 0.35 | 1.91a | 0.01 | 2.01b | 0.02 | 6.22a | 0.01 | 6.24b | 0.02 |
| *E. coli* + Ceftriaxone | 22.03a | 0.25 | 22.49a | 0.27 | 2.11a | 0.01 | 2.19a | 0.02 | 7.02a | 0.02 | 7.45a | 0.03 |
| *E. coli* + Ceftriaxone + Vit E | 22.92a | 0.13 | 23.07a | 0.1 | 2.19a | 0.01 | 2.40a | 0.01 | 7.51a | 0.02 | 7.64a | 0.09 |

Means with different letter in the same column are significantly different at P< 0.05.

ceftriaxone was used as a treatment and could lower morbidity and fatality rates to 10% and 4%, respectively. Likely, ceftriaxone was reported to have potent effects against *E. coli*, *Klebsiella pneumoniae*, *Serratia, Meningococcus* and *Neisseria gonorrhoeae* (Xu *et al*. 2016). Ceftriaxone is a broad-spectrum third generation cephalosporin antibiotic for intravenous or intramuscular injection. It is one of the most used antibiotics due to its high antibacterial potency, wide spectrum of activity and low potential for toxicity (Lee, *et al*. 2009). However, ceftriaxone was used with caution due to its potential adverse effects. In the present study, such adverse effects were investigated. In particular its histopathological effects, induction of inflammatory responses, and alterations in the erythrogram and antioxidant status. The results of the histopathological examination were displayed in Figs. 1-5. The first experimental control group showed normal histological architecture and cellular details of liver, spleen, lung, kidney, and intestine (Fig. 1). The second group which was challenged with *E. coli* with no treatment showed focal aggregation of leucocyte cells and fibroblast in the liver, and focal individualization of some renal tubules separated by mild intertubular edema in the kidney (Fig. 2). While the birds challenged with *E. coli* and received ceftriaxone treatment showed massive focal leukocytic cells infiltrate hepatic parenchyma, diffuse apoptosis, and diffuse caseous necrosis with congestion of blood vessels in the liver. Kidney showed severe congestion of blood vessel with focal intertubular leukocytic cells infiltrate renal medulla, cloudy swelling

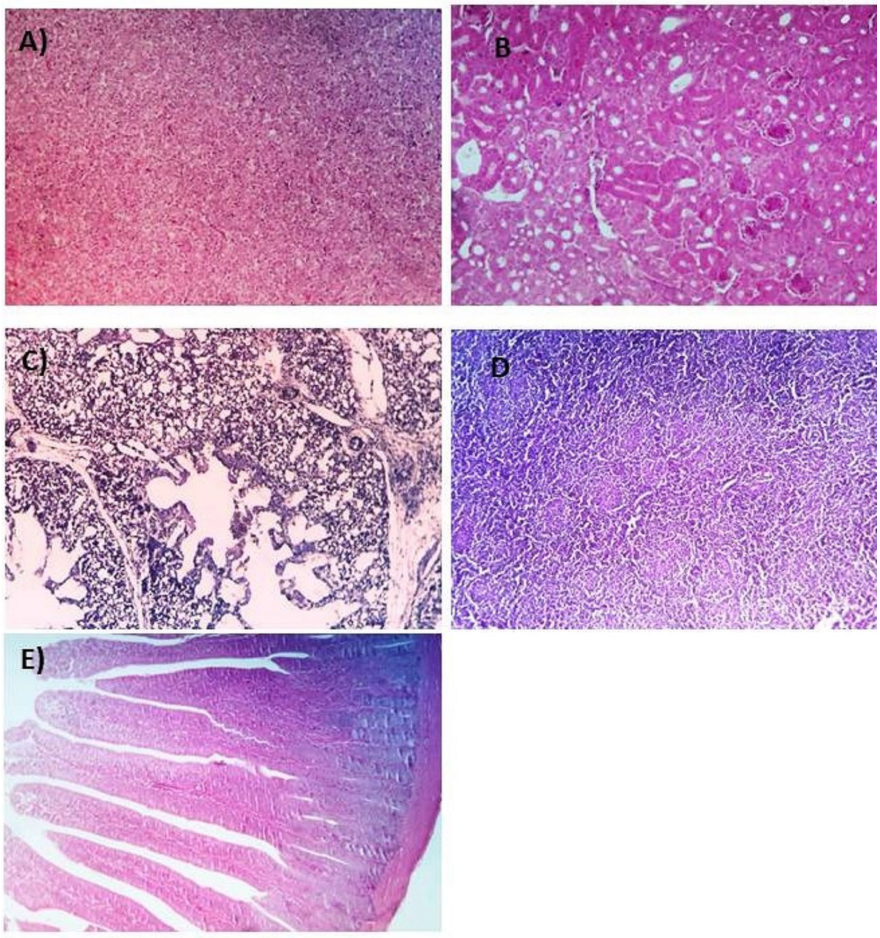


Fig. 1. A) Photomicrograph of liver of Gp1 at the first day post treatment showing normal tissue architecture and cellular details (H&E x 200). B) Photomicrograph of kidney of Gp1 at first day post treatment showing normal renal tubules and glomeruli in the renal cortex (H&E x 200). C) Photomicrograph of lung of Gp1 at the first day post treatment showing normal pulmonary tissue details (H&E x 200). D) Photomicrograph of spleen of Gp1 at first day post treatment showing normal tissue details white and red pulp distribution within parenchyma (H&E x 200). E) Photomicrograph of intestine of Gp1 at the 1st day post treatment showing normal mucosa and submucosa tissues (H&E x 200). F) Photomicrograph of Liver of Gp1 at the 1st day post treatment showing normal tissues of mucosa and submucosa. (H&E x 200).

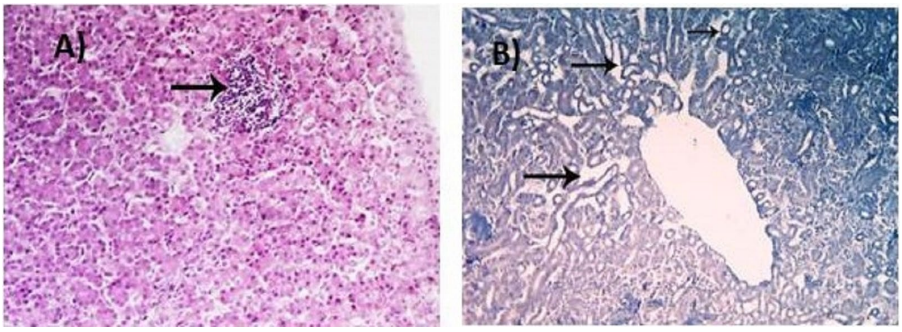


Fig. 2. A) Photomicrograph of the liver in Gp2 at the 7th day post treatment showing focal aggregation of leucocyte cells and fibroblast (arrows) (H&E x 400). B) Photomicrograph of the kidney in Gp2 at the 1st day post treatment showing focal individualization of some renal tubules separated by mild intertubular edema (arrows) (H&E x 400).

of some renal tubules within renal cortex, and focal infiltration of renal cortex with round cells and colonies of *E. coli* rods in addition to intertubular edema (Fig. 3). Lung of group 3 showed perivascular fibrosis with congestion of blood vessel, edema with endotheliosis, and inter alveolar extravasted erythrocytes. Spleen showed perivascular fibrosis, edema with congestion and endotheliosis, and depletion of lymphocytes from white pulp. Intestine of the third experimental group at the first day post treatment showed mucinous degeneration of intestinal villi with partial villus sloughing (Fig. 4). Interestingly, co-treatment of the challenged birds with ceftriaxone, and vitamin E revealed a marked improvement in the histopathological findings as liver showed mild perivascular fibrosis and *E. coli* rods colonization. Kidney showed mild congestion of blood vessels with atrophy of some renal tubules. Spleen showed normal white and red pulps within parenchyma and the intestine showed diffuse congestion of submucosal blood vessels (Fig. 5). In agreement with the obtained results, ceftriaxone caused histopathological alterations in the brain of chicks included clogging, hemorrhage, and inflammatory cell infiltration. When exposed to high quantities, the lesion was more severe, especially in the first 24 hours after exposure (Othman and AL-Zubaidy, 2022). Besides, vitamin E could improve the histopathological lesions in broiler chicken challenged with Newcastle disease virus (Ebrahimzadeh *et al*., 2018).

Erythrogram of the birds challenged with *E. coli* revealed significant decline ($p < 0.05$) in the RBCs, Hb, and PCV% compared with the control. Ceftriaxone treatment could significantly improve the examined blood parameters. Interestingly, adding

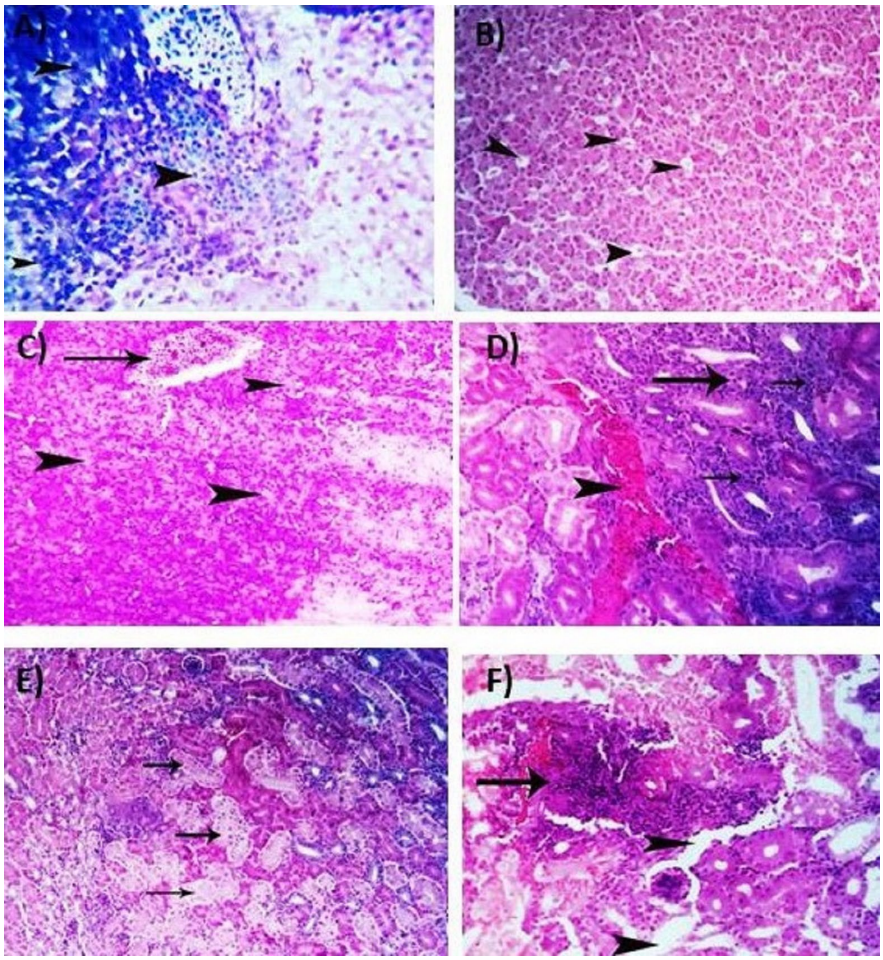


Fig. 3. A) Photomicrograph of the liver in Gp3 at first day post treatment showing massive focal leucocytic cells infiltrate hepatic parenchyma (H&E x 800). B) Photomicrograph of the liver in Gp3 at first day post treatment showing diffuse apoptosis (arrowhead) (H&E x 200). C) Photomicrograph of the liver in Gp3 at the 7th day post treatment showing diffuse caseous necrosis (arrowheads) with congestion of blood vessels (arrow) (H&E x 200). D) Photomicrograph of the kidney in Gp3 at the 1st day post treatment showing severe congestion of blood vessel (arrowhead) with focal intertubular leucocytic cells infiltrate renal medulla (arrows) (H&E x 400). E) Photomicrograph of the kidney in Gp3 at first day post treatment showing cloudy swelling of some renal tubules within renal cortex (arrows) (H&E x 200). F) Photomicrograph of the kidney in Gp3 at the 7th day post treatment showing focal infiltration of renal cortex with round cells and colonies of *E. coli* rods (arrow) in addition to intertubular edema (arrowhead) (H&E x 400).

Table 2. Ameliorative effects of vitamin E. against ceftriaxone-induced alterations in TNF-α and IL-10 in broilers challenged with *E. coli*

| | TNF-α | | | | IL-10 | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st Day | | 3rd Day | | 1st Day | | 3rd Day | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Control | 0.99c | 0.02 | 0.96b | 0.02 | 0.97c | 0.03 | 2.49a | 0.98 |
| *E. coli* | 3.75a | 0.08 | 2.61a | 0.23 | 3.84a | 0.51 | 2.01b | 0.32 |
| *E. coli* + Ceftriaxone | 1.34b | 0.27 | 0.72bc | 0.03 | 1.65b | 0.22 | 2.19b | 0.52 |
| *E. coli* + Ceftriaxone + Vit E | 0.88c | 0.03 | 0.56c | 0.03 | 0.80c | 0.03 | 2.40a | 0.25 |

Means with different letter in the same column are significantly different at $P < 0.05$.

vitamin E to ceftriaxone therapy could cause significant recovery of the blood picture to match the negative control as indicated in Table 1. The obtained results in Table 2 showed that *E. coli* challenge could lead to induction of some inflammatory markers such as TNF-α and IL-10. Ceftriaxone treatment could significantly reduce the upregulated inflammatory markers. Interestingly, adding vitamin E to the treatment protocol could significantly down regulate the inflammatory markers release. Suggesting the beneficial roles of vitamin E in the reduction of the inflammation in *E. coli* challenged birds. The acquired results support the findings of Rao *et al*. (2014), who reported that ceftriaxone caused a considerable drop in IL-10 and TNF-α plasma levels. Additionally, chickens infected with *E. coli* displayed an increase in liver IL-10, according to Abd-El Rhman *et al*. (2018). In addition, Elnagar *et al*. (2021) reported that ileal IL-10 levels in *E. coli* showed a substantial rise. Ceftriaxone's protective properties are in line with those described by Arhoumah (2018), who found that cefepime at a dose of 45 mg/kg bwt considerably lessened the changes in TNF-α and IL-10. Moreover, Vitamin E -tocotrienol blocks TNF-α stimulated NF-B activation by increasing anti-inflammatory A20 through sphingolipid modulation, including elevation of intracellular dihydroceramides (Yang and Jiang, 2019). This study further investigated how the treatment with ceftriaxone and/or *E. coli* affected the birds' antioxidant level. In comparison to control broilers, the acquired data showed that ceftriaxone significantly reduced serum Gpx, SOD, and MDA. The serum levels of Gpx and SOD significantly decreased in *E. coli*-infected birds, while MDA levels increased. Broilers infected with *E. coli* and given both cef-

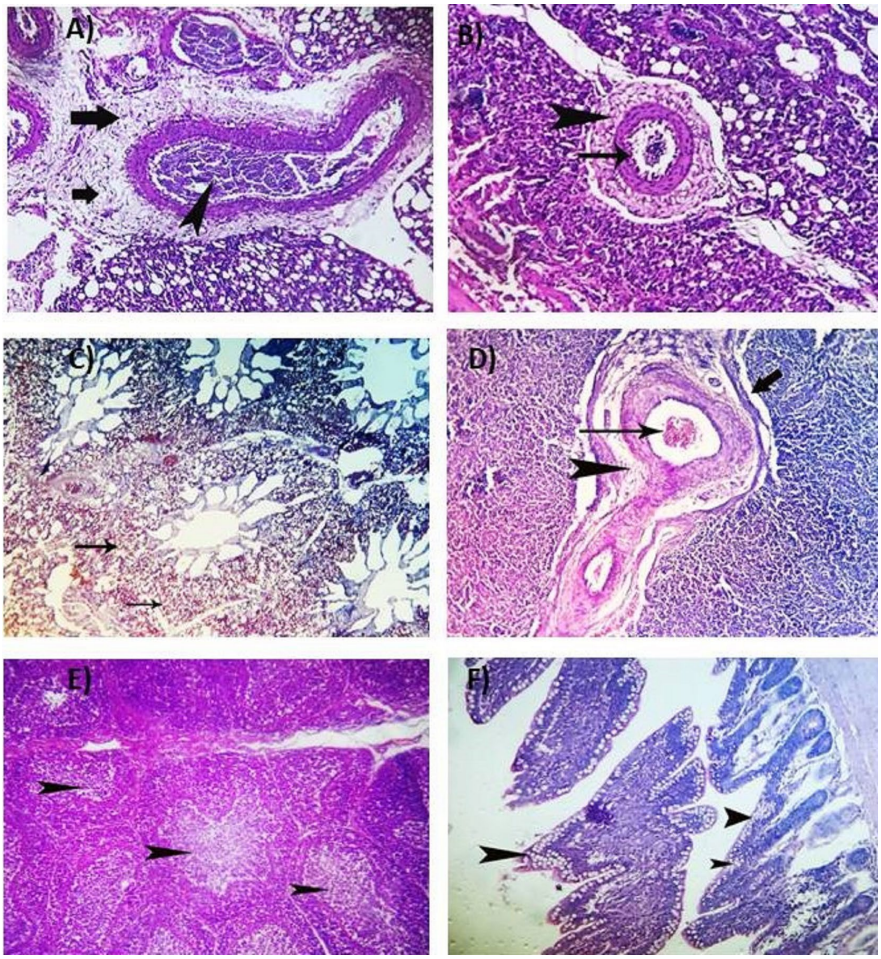


Fig. 4. A) Photomicrograph of the lung in Gp3 at first day post treatment showing perivascular fibrosis (thick arrows) with congestion of blood vessel (arrowhead) (H&E x 400). B) Photomicrograph of the lung in Gp3 at first day post treatment showing perivascular fibrosis (thick arrows) and edema with endotheliosis (thin arrow) (H&E x 400). C) Photomicrograph of the lung in Gp3 at the 7th day post treatment showing inter alveolar extravasted erythrocytes (arrows) (H&E x 400). D) Photomicrograph of the spleen in Gp3 at the 1st day post treatment showing perivascular fibrosis (arrowhead) and edema (thick arrow) with congestion (thin arrow) and endotheliosis (H&E x 400). E) Photomicrograph of the spleen in Gp3 at the 7th day post treatment showing depletion of lymphocytes from white pulp (arrowhead) (H&E x 200). F) Photomicrograph of the intestine in Gp3 at the first day post treatment showing mucinous degeneration of intestinal villi (arrowhead) with partial villus sloughing (H&E x 400 ).

Table 3. Ameliorative effects of vitamin E against ceftriaxone-induced alterations SOD, GPx, and MDA in broilers challenged with *E. coli*

| | SOD | | | | | | GPx | | | | | | MDA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st Day | | 7th Day | | 14th day | | 1st Day | | 7th Day | | 14th day | | 1st Day | | 7th Day | | 14th day | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Control | 98a | 4.18 | 96.8a | 2.28 | 92.8a | 3.7 | 115.2a | 3.56 | 119.8a | 3.03 | 118.8a | 2.58 | 5.99b | 0.71 | 6.09b | 0.13 | 5.98b | 0.22 |
| *E. coli* | 63.6c | 7.73 | 87b | 3.74 | 83b | 5.78 | 100.4b | 2.96 | 93.4c | 4.03 | 96.2b | 5.26 | 10.48a | 0.78 | 10.69a | 0.08 | 10.91a | 0.36 |
| *E. coli* + Ceftriaxone | 82b | 7.48 | 92ab | 2.11 | 87.8ab | 7.66 | 105.2ab | 2.94 | 108aab | 4.06 | 106.2ab | 4.61 | 9.62a | 0.59 | 10.99a | 0.39 | 6.46b | 0.12 |
| *E. coli* + Ceftriaxone + Vit E | 93.8a | 1.48 | 98.6a | 1.14 | 91.2a | 1.3 | 107.8ab | 3.34 | 114.6a | 1.14 | 111.2a | 1.31 | 8.35ab | 0.14 | 6.81b | 0.12 | 5.92b | 0.05 |

Means with different letter in the same column are significantly different at P< 0.05.

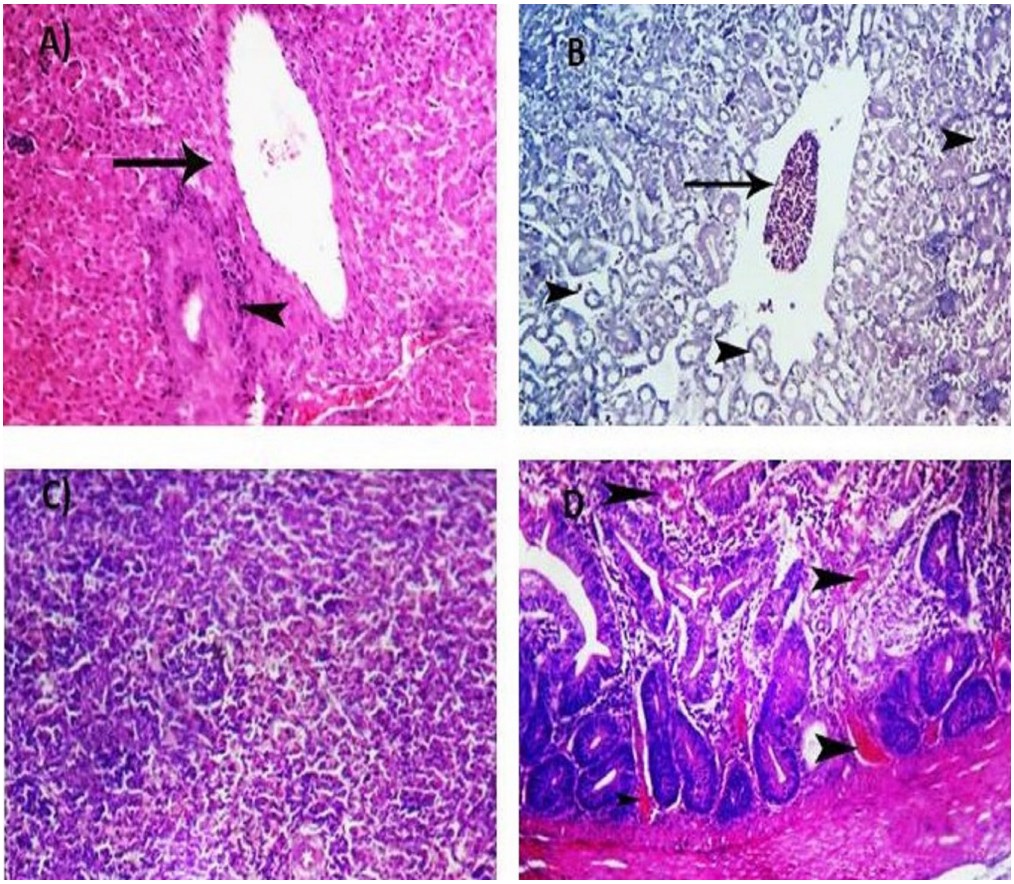


Fig. 5. A) Photomicrograph of the liver in Gp4 at the 7th day post treatment showing mild perivascular fibrosis (arrow) and *E. coli* rods colonization (arrowhead) (H&E x 400). B) Photomicrograph of the kidney in Gp4 at first day post treatment showing mild congestion of blood vessels (arrow) with atrophy of some renal tubules(arrowhead) (H&E x 400). C) Photomicrograph of the spleen in Gp4 at first day post treatment showing normal white and red pulps within parenchyma (H&E x 200). D) Photomicrograph of the intestine in Gp4 at first day post treatment showing diffuse congestion of submucosal blood vessels (arrowhead ) (H&E x 400).

triaxone and vitamin E, however, demonstrated a considerable rise in serum Gpx and SOD in addition to a fall in MDA (Table 3). These outcomes were consistent with those reported by Khaled *et al*. (2014), who found that ceftriaxone caused a rise in serum MDA along with a notable decline in SOD and Gpx. Dayana and Manasa (2020) verified that ceftriaxone caused lipid peroxidation and changed the activity of antioxidant enzymes, corroborated these findings. In addition, *E. coli* infection caused an imbalance between oxidants and antioxidants, which lowered levels of SOD and Gpx (El-Kilany *et al*., 2018). Also, Ashraf *et al*. (2019) noted an improvement in Gpx, SOD, and MDA in *E. coli*-infected grill treated with another cephalosporin (cephradine). Broiler chicken meals supplemented with 200 mg/kg of vitamin E had the greatest impact on the birds' overall antioxidant status, blood antioxidant enzyme activity, and vitamin E concentrations in the liver and breast muscles.

## CONCLUSION

The obtained results of the present study demonstrated that ceftriaxone is an effective antimicrobial medication, particularly against *E. coli* in broilers. In order to reduce ceftriaxone-induced adverse effects in broilers, it is highly recommended to use vitamin E.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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