Indian Journal of Animal Sciences **96** (2): 103–109, February 2026/Article
https://doi.org/10.56093/ijans.v96i2.175877

Adjunctive effect of ascorbic acid on tetracycline treatment in broiler chickens challenged with tetracycline-resistant *Escherichia coli*

PAVITHRA M[1], RAMASAMY THANGAMALAI[2✉], RAMESH S[3], ELAMARAN A[4], JAIKANTH C M[5], M AKSHAY DURGA PRASAD[6] and SAMUEL MASILAMONI RONALD[7]

Tamil Nadu Veterinary and Animal Sciences University, Chennai Tamil Nadu 636 112 India

Received:19 February 2026; Accepted: 11 June 2026

ABSTRACT

The emergence of multidrug-resistant organisms due to the indiscriminate use of antibacterials has become a critical health concern for humans and animals. This study aimed to explore the potential of ascorbic acid to enhance the *in vitro* and *in vivo* effectiveness of tetracycline against Avian Pathogenic *Escherichia coli* (APEC) infections resistant to tetracycline. Isolates of *E. coli* from broiler chickens affected by colibacillosis were screened for the presence of tetracycline-resistant genes. The *in vitro* antibacterial properties of ascorbic acid were evaluated. In the *in vivo* experiment, the birds were pre-treated with ascorbic acid beginning on day three, and on day seventeen of trial, they were challenged with tetracycline-resistant APEC. Clinical symptoms of infection were observed two days post-challenge and then treated with tetracycline for 5 days continuously. After five days of tetracycline treatment, blood samples and organ tissues were collected for haematological, biochemical and histopathological analyses. The study revealed that 60% of prevalence rate for APEC isolates showing the highest resistance with tetracycline. The combination of ascorbic acid did not markedly enhance the antibacterial efficacy of tetracycline *in vitro*, its administration *in vivo* has notably diminished disease severity. Although no marked in vitro synergistic effect was observed, ascorbic acid supplementation was associated with reduced disease severity *in vivo*, suggesting a potential supportive role during antibacterial therapy that warrants further investigation.

Keywords: Ascorbic acid pretreatment, Broiler chicken, Tetracycline Resistance, *tet* A gene, *tet* B gene

Antibacterials are commonly used in poultry industry to treat various bacterial infections and for prophylactic purposes. This has garnered resistance in both commensal and pathogenic *E. coli* due to the selection pressure of antibacterials. The emergence of Multidrug Resistance (MDR) organisms has become a major health threat to human and animals. The prevalence of MDR in avian pathogenic *E. coli* (APEC) is reported to be 81% with 45% capable biofilm formation (Goudarztalejerdi *et al*. 2022)

Tetracycline is one of the most used antibacterial agents in poultry for various diseases either in feed or in water, majorly due to its broad spectrum of activity, low cost and easy availability. Tetracycline is also the most widely detected antimicrobial agent in poultry manure with an average content of 5.16 mg/kg (Topi *et al*. 2020) indicating its widespread usage in poultry farming.

Due to lack of stewardship for antimicrobial usage in poultry, antimicrobial resistance (AMR) has become a major health threat. The World Health Organization (WHO) and Food & Agriculture Organization (FAO) had jointly established the National Action Plan – Antimicrobial Resistance which is being coordinated by the National Centre for Disease Control. Various approaches were overcome the AMR include vaccination against diseases, immunomodulation, replacing antibiotics with pro and prebiotics in feeds, combination therapy, use of engineered bacteriophages and various natural compounds as antibacterial resistance breakers (Rahal *et al*. 2021).

Ascorbic acid is a well-known nutritional supplement with rich antioxidant properties. It is also found to possess antibacterial potency under *in vitro* conditions (Hassuna *et al*. 2023). Based on the above, the present study was carried out to evaluate the ability of ascorbic acid to modulate the *in vitro* and *in vivo* efficacy of tetracycline against tetracycline resistant *E. coli* both *in vitro* and in experimentally induced tetracycline-resistant colibacillosis in broiler chicken.

MATERIALS AND METHODS

Sample collection: A total of sixty liver swabs were collected aseptically from moribund broiler chicken, suspected to be affected by colibacillosis from various integrated broiler chicken farms in tropical regions of

Present address: [1]PG Scholar. [2]Associate Professor and Head. [3]Professor and Head, [4]Assistant Professor, [5]Assistant Professor.[6]PG Scholar, Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 007, India. [7]Professor, Department of Veterinary Microbiology, Madras Veterinary College, Chennai 600 007. ✉Corresponding author email: drrams19@gmail.com

Southern India (Tamil Nadu) and were stored at 4° C until further use.

Isolation and identification of APEC: APEC was isolated using MacConkey agar and Eosin Methylene Blue (EMB) agar after enrichment of liver swabs with nutrient broth. The presumptive APEC colonies were subjected to gram staining for identification of gram-negative bacteria and biochemical tests were carried out using HiIMViC™ test kit. Molecular confirmation of *E. coli* was also done by PCR amplification targeting *Adk* gene.

DNA extraction: A single loop of bacterial colony was added to 200 µl of Nuclease Free Water (NFW) and boiled at 100°C for 10 minutes in water bath and was chilled immediately to -20°C. Then centrifugation was done at 6300 x g for 2 minutes (Fang and Hedin, 2003).

Antimicrobial susceptibility testing - Disc diffusion test: Thirty six APEC isolates were subjected to a disc diffusion test to study their sensitivity pattern to different antibacterials on Mueller Hinton Agar plates as per CLSI standards. The antibacterials used in this study were Amoxycillin–Clavulanate (30 mcg), Cefotaxime (30 mcg), Ceftazidime (30 mcg), Cephalexin (30 mcg), Tetracycline (30 mcg), Cotrimoxazole (25 mcg), Neomycin (10 mcg), Gentamicin (10 mcg), Levofloxacin (5 mcg), Colistin (10 mcg), Cefotaxime + clavulanic acid (30/10 mcg) from HiMedia™.

PCR analysis: The analysis is carried out in a conventional PCR. The primer sequence for *Adk* gene for *E. coli* confirmation and tetracycline efflux genes - *tetA, tetB* used in this study are listed below in Table 1.

The 20 µl reaction mixture for PCR amplification contained 10 µl of GeNei red dye master mix, 1 µl of forward and reverse primer each, 2 µl of DNA template and 6 µl of nuclease free water. The cyclical condition for amplification of each gene is given in Supplementary Table 1.

Estimation of Minimum Inhibitory Concentration (MIC): The MIC of tetracycline (M/s. Sigma Aldrich) and its combination with ascorbic acid (L-ascorbic acid from M/s. SRL chem) against APEC was estimated using both macro broth dilution method (CLSI M07 standards, 2018) and microbroth dilution method using resazurin dye test.

In vivo study: In vivo experimental study was conducted using broiler chicken with the approval of Institutional Animal Ethics Committee (No: 873/DFBS/IAEC/2021, dated: 25.08.2021).

Table 1. The forward and the reverse nucleotide sequence for the primers used in the study

Target gene	Sequence (5' to 3')	Size (bp)
Adk	F: ATTCTGCTTGGCGCTCCGGG R: CCGTCAACTTTCGCGTATTT	590
tet (A)	F: GGTTCACTCGAACGACGTCA R: CTGTCCGACAAGTTGCATGA	577
tet (B)	F: CCTCAGCTTCTCAACGCGTG R: GCACCTTGCTCATGACTCTT	634

Experimental birds: Eighty day-old broiler chicks of Vencobb strain purchased from M/s. Venkateshwara hatcheries, Hyderabad, India were used for this study. Broiler chicks were randomly divided into five groups (Table 2) with sixteen broiler chicks per group.

From day 3, Group 1, 2, and 3 birds were fed control diet whereas, Group 4 and 5 birds were fed feed containing ascorbic acid at the rate of 200 mg/kg of feed (M/s. Optima Poultry Pvt. Ltd, Coimbatore, Tamil Nadu, India).

Challenge procedures: On seventeenth day (fourteenth day since continuous ascorbic acid supplementation), broiler chickens were infected with tetracycline resistant APEC. For infecting birds, APEC was grown in Brain Heart Infusion agar overnight at 37° C. All the birds except first group were challenged orally with 8 ml of overnight grown tetracycline resistant *E. coli* culture containing 1.2×10^9 CFU/ml (Bonassa *et al.* 2021). The birds in groups 3 and 5 were treated with tetracycline hydrochloride through drinking water (M/s. MSD Animal Health) for 5 days at the dose rate of 100 mg/kg of body weight after the observation of clinical signs. Birds were sacrificed after five days of tetracycline treatment by cervical dislocation and necropsy was carried out to estimate the severity of the disease. The body weights of the birds were taken on days 0, 3 and 6 post-infection.

Samples collected: Terminal blood and tissue samples were collected for hematological and histopathological examination. Serum was obtained and stored at - 20°C. Tissue specimens such as liver, heart, intestine, spleen and kidney were collected and fixed in 10% neutral buffered formalin for further histopathological examination.

Statistical analysis: Data were analyzed using GraphPad InStat version 6.01 for Windows (GraphPad Software, San Diego, California, USA). Results are expressed as Mean ± SE. The minimum inhibitory concentration (MIC) data were analyzed using an unpaired Student's t-test. Biochemical and hematological parameters were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Body weight measurements recorded at different time points were analyzed using

Table 2. Experimental design

Group	Treatment	No. of Birds
1	Healthy Control	16
2	Infected control (*E. coli*)	16
3	Infected + Tetracycline (100 mg/kg of body weight)	16
4	Infected + Ascorbic acid (200 mg/kg of feed)	16
5	Infected + Ascorbic acid (200 mg/kg of feed) + Tetracycline (100 mg/kg of body weight)	16
	Total	80

repeated-measures ANOVA. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Prevalence rate: Sixty liver swabs were collected from birds which were suspected to have died due to *E. coli* infection. The prevalence rate of *E. coli* was found to be 85% based on colony morphology. The biochemical tests and molecular confirmation for the *Adk* gene (Supplementary Figure 1a) yielded 60% prevalence, which is consistent with the findings of Sarker (2019).

Antimicrobial sensitivity test: Maximum resistance was observed against tetracycline (69.44%), cotrimoxazole (66.67%), and levofloxacin (66.66%) followed by neomycin (47.22%), cephalexin (16.67%), and a higher degree of susceptibility was observed against colistin and ceftazidime (91.67%), cefotaxime (88.89%) and gentamicin (86.11%) (Figure 1). Among the samples, 66.67% were found to be resistant to at least 3 antimicrobial agents screened phenotypically. These findings were in close agreement with the observations of Saha *et al.* (2020). Higher resistance to tetracycline and levofloxacin might be due to their frequent and indiscriminate use in poultry farms to prevent disease outbreaks (Bhardwaj *et al.* 2021). In the double disc diffusion method, 8.33% of the samples were found to be positive for ESBL which was lower than the recent report published by Grakh *et al.* (2022).

Tetracycline efflux genes: The *tet* genes are involved in the efflux of tetracyclines from bacterial cells. PCR was done to study the presence of *tetA* and *tetB* genes (Supplementary Figure 1b and 1c) and the recovery rates were found to be 56.25% and 21.88% respectively, which is similar with the study of Bhardwaj *et al.* (2021). These differences might be due to differences in usage patterns of antimicrobial agents in veterinary and human medicine. Among these, 9.37% of isolates were positive for both efflux genes.

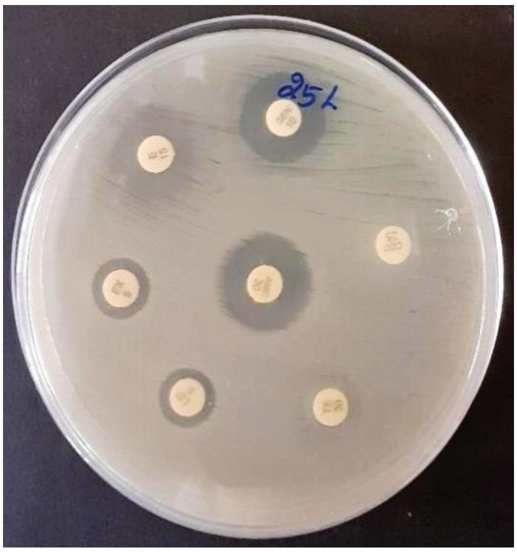

Fig.1. ABST by disc diffusion method for various antibacterial drugs

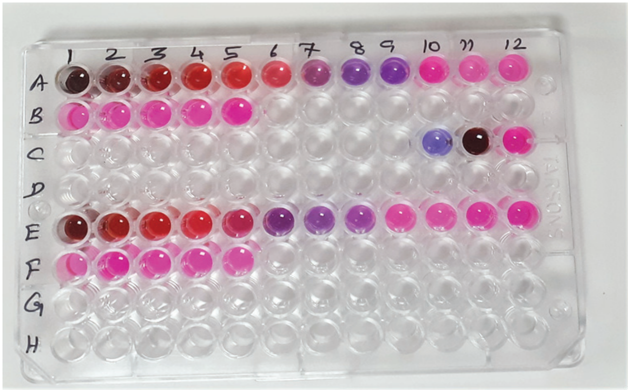

Fig. 2. MIC of Tetracycline and its combination with ascorbic acid

Minimum inhibitory concentration: The MIC of tetracycline against isolated pathogenic *E. coli* was found to be 456.10 ± 65.22 µg/ml but when combined with ascorbic acid the MIC reduced to 373 ± 44.33 µg/ml. The MIC of tetracycline was found to be higher when compared with the previous studies (Ramasamy *et al.* 2021). The increased MIC value of tetracycline might be due to the impact of the continuous usage of tetracyclines in livestock for prophylactic and therapeutic purposes (Figure 2).

Ascorbic acid in vitro antibacterial effect: Ascorbic acid showed no antibacterial activity against *E. coli*, in disc diffusion and macro dilution method, both alone and in combination with tetracycline. Shivaprasad *et al.* (2021) reported that ascorbic acid had dose-time-dependent antibacterial activity against *E. coli.* Some earlier works had reported that ascorbic acid enhanced the effects of antimicrobial agents against *Pseudomonas,* and *Escherichia coli* organisms in *in-vitro* studies (Abdelraheem *et al.* 2022; Hassuna *et al.* 2023, Sriraam *et al.* 2022).

Clinical signs and body weight: The clinical signs observed in the infected birds were conjunctivitis, ruffled feathers, increased body temperature and watery faeces. No mortality was observed. There was no significant difference ($p < 0.05$) in the body weight of the birds of various groups.

Hematological profile: Hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC) in birds of all groups did not differ significantly ($p > 0.05$) between the groups.

The values were increased non-significantly in the infected group birds when compared to the control group. One of the recent studies also noticed a rise in haemoglobin and hematocrit levels in *E. coli* infection (Rosa *et al.* 2020). This polycythemia condition may be due to dehydration in infected birds caused by diarrhoea. The higher WBC counts in the infected group of birds could be related to an acute inflammatory reaction against *E. coli* and antigenic stimulation of the host immune system (Jaensch *et al.* 2004) (Table 3).

Biochemical parameters: The results of serum biochemical parameters were shown in the Table 4.

Table 3. Effect of tetracycline on hematological parameters in *E. coli* challenged birds fed with ascorbic acid supplemented diet

Parameter	Group I	Group II	Group III	Group IV	Group V	P value
Hemoglobin (mg/dl)	10.36 ± 1.748	12.04 ± 0.253	9.886 ± 1.668	8.514 ± 2.223	10.71 ± 1.915	0.7505^{ns}
PCV (%)	25.10 ± 4.384	27.36 ± 0.8121	23.39 ± 3.980	20.69 ± 5.418	25.67 ± 4.801	0.8886^{ns}
RBC (x10^{6}µL)	4.686 ± 0.7989	5.583 ± 0.2384	4.396 ± 0.7879	3.961 ± 1.033	4.651 ± 0.7815	0.6877^{ns}
WBC (x10^{3}µL)	20857 ± 3744	26000 ± 2093	22857 ± 3912	15286 ± 4324	21286 ± 3784	0.5046^{ns}

Means ± SE bearing same superscripts in between the columns does not differ significantly at 5% level.

Table 4. Effect of tetracycline on serum biochemical parameters in *E. coli* challenged birds fed with ascorbic acid supplemented diet

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p value
Total protein (g/l)	3.029 ± 0.143^{b}	4.110 ± 0.131^{a}	3.192 ± 0.121^{b}	3.343 ± 0.093^{b}	3.318 ± 0.133^{b}	<0.001
Albumin (g/l)	2.090 ± 0.233^{a}	2.280 ± 0.051^{a}	1.627 ± 0.041^{ab}	1.900 ± 0.095^{a}	1.485 ± 0.062^{b}	<0.001
Globulin (g/l)	1.371 ± 0.127	1.830 ± 0.134	1.509 ± 0.136	1.443 ± 0.127	1.654 ± 0135	0.1847^{ns}
ALT (IU/L)	20 ± 0.799^{a}	18.3 ± 1.126^{ac}	14.27 ± 1.287^{bc}	13 ± 1.094^{b}	18.62 ± 1.233^{a}	<0.001
ALP (IU/L)	1853 ± 55.15	2269 ± 136.2	2084 ± 86.86	1904 ± 87.66	2173 ± 105.8	0.0435^{ns}
Uric acid (mg/dl)	6.210 ± 0.453	5.671 ± 0.577	4.821 ± 0.479	5.604 ± 0.387	5.455 ± 0.369	0.3918^{ns}

Value is expressed in Mean ± SE, bearing same superscripts in between the columns do not differ significantly at 5% level.

The estimated total serum protein was found to be significantly ($p<0.001$) increased in group 2 in comparison with other groups, which agrees with a recent study (Rosa *et al.* 2020). The albumin level was declined non-significantly in group 3 (tetracycline treated) and 5 (tetracycline + ascorbic acid) birds than in group 2 birds. This result was found to be consistent with the study reported by Rosa *et al.* (2020). The decreased albumin levels in other infected groups could be associated with hepatocellular and tubular epithelial cell damage during the detoxification process of *E. coli* bacterial toxins. ALT level was found to be significantly ($p<0.001$) decreased in groups 3 and 4 when compared to control. However, previous studies noticed an increase in serum ALT levels in colibacillosis-affected birds which is contradictory to our observations (Amin *et al.* 2020). There was no significant difference in the globulin, ALP and uric acid levels between groups.

Gross pathology: The birds challenged with *E. coli* showed cloudy air sacs slightly, enlarged, rounded and discolored liver with mild focal haemorrhages on the dorsal aspect. The heart showed chalky fibrinous depositions on entire surface with adherence to the thoracic ribs. Mild enlargement was noticed in the spleen of these birds with polyserositis and hemorrhagic mucosal damage in intestines. These results are in concurrence with the reports of Surjagade *et al.* (2020) and Awaad *et al.* (2021) who had recorded air sacculitis, pericarditis, perihepatitis, discolouration of the liver and hydropericardium of different degrees in APEC infected broiler chicken.

The gross pathology of the organs of birds treated individually with tetracyclines (Group 3) and Ascorbic acid (Group 4) fared better than the positive control. However, the birds which received both tetracyclines and Ascorbic acid exhibited significant reconstruction in the organ tissue. These results coincided with the works of Khaldoun Oularbi *et al.* (2017) and El-Shafei *et al.* (2017) who reported that ascorbic acid treatment reduced the hepatotoxic and nephrotoxic effects in rats. The results of various organs were shown in supplementary Figure 3.

Histopathology: On histopathological examination, degenerative changes were seen in *E. coli* challenged

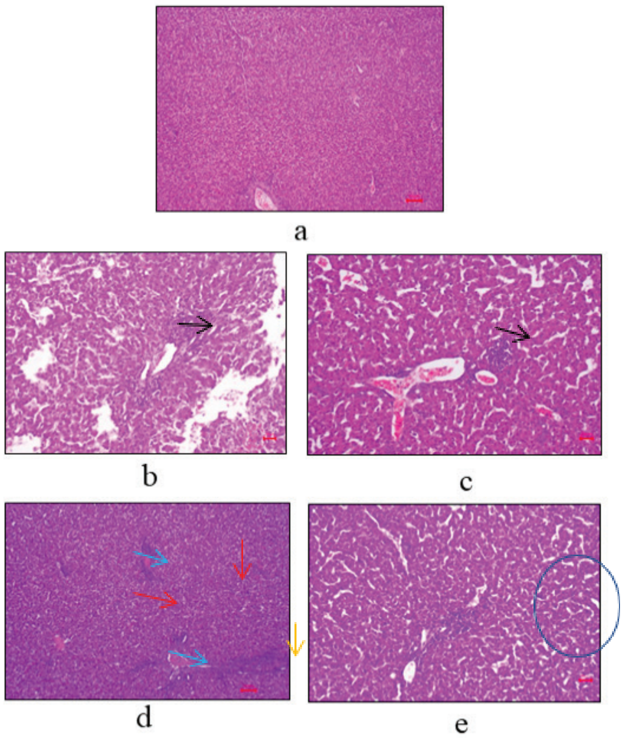

Fig. 3. Histopathology of Liver (a) Group 1 - No abnormalities detected (NAD), (b) Group 2 - Congestion, degeneration and periportal MNC infiltration (Arrow), (c) Group 3 - Congestion, mild degeneration of hepatocytes and mild periportal MNC infiltration (Arrow), (d) Group 4 – Congestion (blue arrow), mild degeneration (Red arrow) and minimal MNC infiltration (Yellow arrow), (e) Group 5 - Mild degeneration (Circle)

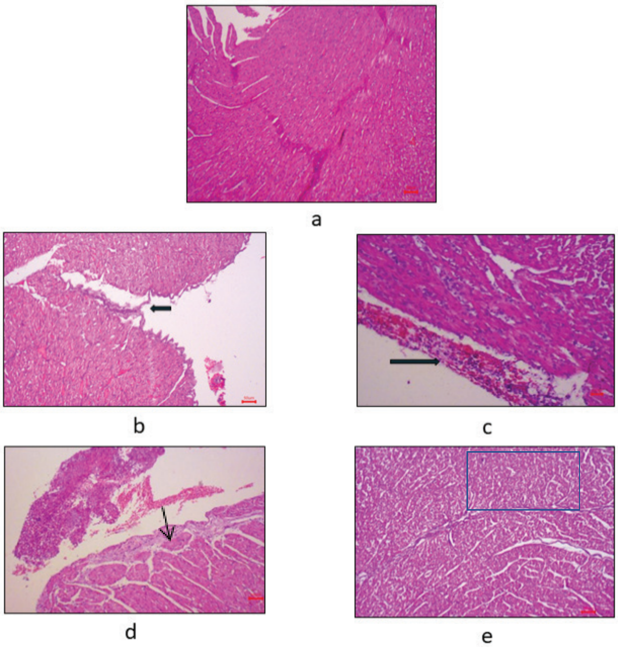

Fig. 4. Histopathology of Heart (a) Group 1 - NAD, (b) Group 2 - mild fibrinous exudate with myocardial degeneration and necrosis with heterophilic infiltration (Arrow), (c) Group 3 - Pericardial congestion and haemorrhage, myocardial degeneration (Arrow), (d) Group 4 - Fibrinous exudate, myocardial degeneration and haemorrhage (Arrow), (e) Group 5 - Mild myocardial degeneration (Rectangle)

birds (Group 2). The liver of these birds showed severe infiltration of mononuclear cells (MNC) in the perivascular and periportal areas of hepatic vessels, haemorrhages between the hepatic cells and marked hepatocellular degeneration (Figure 3b). The hearts revealed mild eosinophilic fibrinous exudate in the myocardium with severe myocardial degeneration and inflammatory cell infiltration. In addition, they also recorded clumps of bacterial organisms in the myocardium (Figure 4b). The spleen revealed thickening and detachment of the splenic capsule with enlargement of the capsular artery (Figure 5b) in comparison to the control (Figure 5a). The kidneys showed moderate tubular epithelial cell degeneration and multifocal moderate interstitial MNC infiltration (Figure 6b). The intestines revealed stunting of villi, congestion, mild degeneration and necrosis of mucosal epithelium of villi (Figure 7b). These histopathological changes were in concurrence with the previous studies (Hashem *et al*. 2019; Awaad *et al*. 2021).

There was a generalized decrease in the severity of histopathological lesions in the organs of birds supplemented with tetracycline hydrochloride and ascorbic acid (Group 5) in combination when compared with other groups. The histopathological lesions were shown in Figure 3, 4, 5, 6 and 7. These results coincided with the previous study reported by Mousavi *et al.* (2020) who concluded that the ascorbic acid supplementation improved the symptoms of campylobacteriosis in mice models and this alleviation

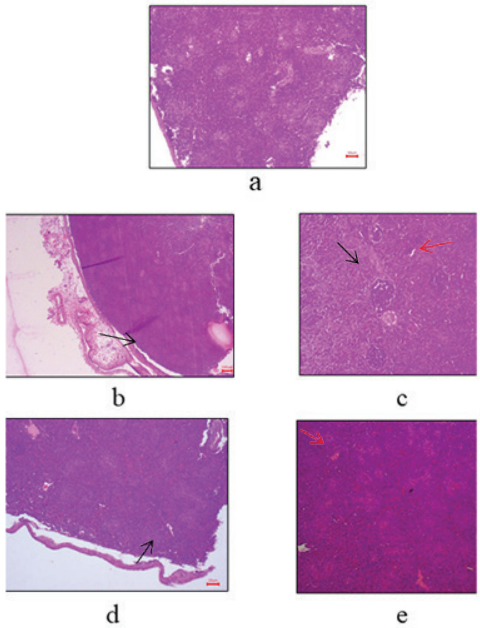

Fig. 5. Histopathology of Spleen (a) Group 1 - NAD, (b) Group 2 - Thickened and detached splenic capsule with fibrino-cellular exudate, splenic capsular artery thickened and dilated (Arrow), (c) Group 3 - mild congestion (Red arrow) and lymphoid cell depletion (black arrow), (d) Group 4 - Congestion and thickening of splenic capsule, (e) Group 4 – Congestion (Red arrow)

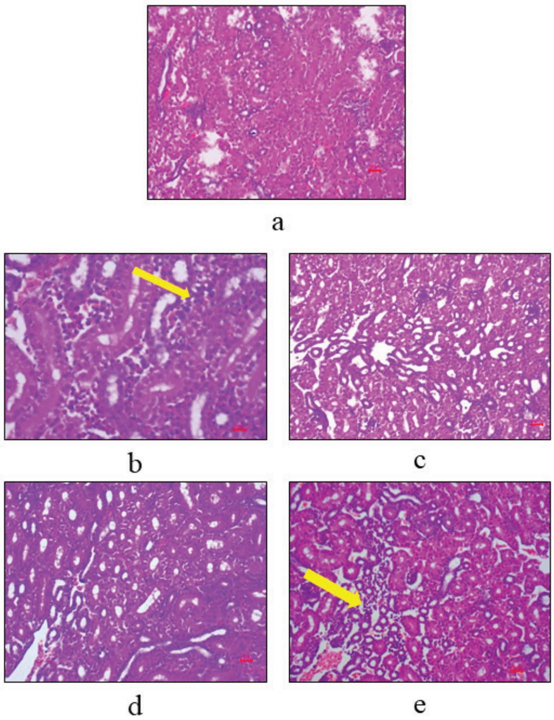

Fig. 6. Histopathology of Kidney (a) Group 1 - NAD, (b) Group 2 - Multi focal mild to moderate tubular epithelial degeneration, congestion, mild interstitial MNC and heterophilic infiltration (Yellow arrow), (c) Group 3 and (d) Group 4 - mild tubular epithelial cell degeneration (Red arrow), (e) Group 5 – Congestion (Red arrow) and heterophilic infiltration (Yellow arrow)

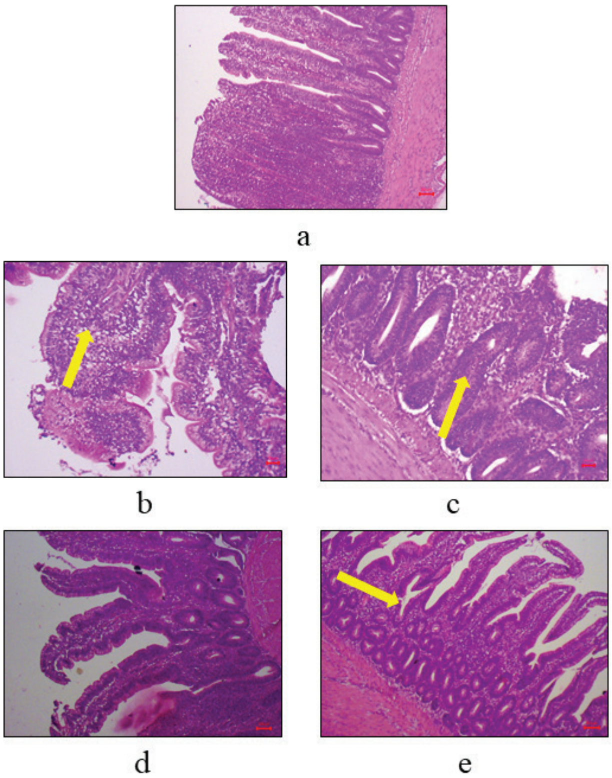

Fig. 7. Histopathology of Intestine (a) Group 1 - NAD, (b) Group 2 - mild degeneration and necrosis of mucosal epithelium of villi with MNC infiltration (Yellow arrow), (c) Group 3 - distorted crypt epithelial cell hyperplasia, congestion and mild hyperplasia of goblet cell (Yellow arrow), (d) Group 4 - NAD, (e) Group 5 - minimal MNC filtration in the mucosa (Yellow arrow)

in disease severity may be due to its anti-inflammatory, antioxidant and immune modulation properties of ascorbic acid.

The present study demonstrated that ascorbic acid did not markedly enhance the *in vitro* antibacterial activity of tetracycline against tetracycline-resistant Avian Pathogenic *Escherichia coli* (APEC). However, under *in vivo* conditions, ascorbic acid supplementation was associated with reduced disease severity and improved gross and histopathological outcomes in experimentally infected broiler chickens. These findings suggest that ascorbic acid may serve as a supportive adjunct to antibacterial therapy, potentially through biological and pharmacological mechanisms beyond direct antibacterial activity. Further studies are warranted to elucidate the underlying mechanisms and to determine its practical application in the management of antimicrobial-resistant bacterial infections in poultry.

ACKNOWLEDGEMENT

The authors acknowledge the Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India for funding.

REFERENCES

Abdelraheem W M, Refaie M M, Yousef R K M, Abd El Fatah A S, Mousa Y M and Rashwan R. 2022. Assessment of antibacterial and anti-biofilm effects of vitamin C against Pseudomonas aeruginosa clinical isolates. *Frontiers in Microbiology* **13**: 847449.

Amin U, Kamil S A, Wani B M, Qureshi S, Shah S A, Dar T A and Mir M S. 2020. Haematological and biochemical alterations of broiler chicken. affected naturally with colibacillosis. *International Journal of Current Microbiology and Applied Sciences* **9**: 1906–1913.

Awaad M H H, El-Moustafa K M, Zoulfakar S A, Elhalawany M S, Mohammed F F, El-Reay R M and Morsy E A. 2021. The role of silver nanoparticles in the reluctance of colisepticemia in broiler chickens. *Journal of Applied Poultry Research* **30**: 100155.

Bhardwaj K, Suchitra S M, Baliga S, Unnikrishnan B, Shantharam B and Shetty V K. 2021. Research Note: Characterization of antibiotic-resistant phenotypes and linked genes of *Escherichia coli* and *Klebsiella pneumoniae* from healthy broiler chickens. Karnataka, India. *Poultry Science* **100**: 101094.

Bonassa K P, Miragliotta M Y, Simas R C, Eberlin M N, Anadón A, Moreno R A and Reyes F G. 2021. Pharmacokinetics, Pharmacodynamic efficacy prediction indexes and Monte Carlo Simulations of Enrofloxacin hydrochloride against bacterial strains that induce common clinical diseases in broiler chickens. *Frontiers in Veterinary Science* **7**: 606872.

El-Shafei R A and Saleh R M. 2016. Pharmacological effects of vitamin C & E on diclofenac sodium intoxicated rats. *Biomedicine and Pharmacotherapy* **84**: 314–322.

Fang H and Hedin G. 2003. Rapid screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time PCR assay. *Journal of Clinical Microbiology* **41(7)**: 2894–2899.

Goudarztalejerdi A, Mohammadzadeh A, Niazi K and Mohammad Mirzaei M. 2022. High prevalence of multidrug resistance and biofilm-formation ability among avian *Escherichia coli* isolated from broilers in Iran. *Microbial Drug Resistance* **28**: 244–254.

Grakh K, Mittal D, Prakash A and Jindal N. 2022. Characterization and antimicrobial susceptibility of biofilm-producing Avian Pathogenic *Escherichia coli* from broiler chickens and their environment in India. *Veterinary Research Communications* **46**: 537–548.

Hashem M A, Neamat-Allah A N, Hammza H E and Abou-Elnaga H M. 2019. Impact of dietary supplementation with *Echinacea purpurea* on growth performance, immunological, biochemical, and pathological findings in broiler chickens infected by pathogenic *E. coli*. *Tropical Animal Health and Production* **52**: 1599–1607.

Hassuna N A, Rabie E M, Mahd W K M, Refaie M M, Yousef R K M and Abdelraheem W M. 2023. Antibacterial effect of vitamin C against uropathogenic *E. coli in vitro* and *in vivo*. *BMC Microbiology* **23**(1): 112.

Jaensch S and Clark P. 2004. Haematological characteristics of response to inflammation or traumatic injury in two species of black cockatoos: *Calyptorhynchus magnificus* and *C. funereus*. *Comparative Clinical Pathology* **13**: 9–13.

Khaldoun Oularbi H, Richeval C, Lebaili N, Zerrouki-Daoudi N, Baha M, Djennas N and Allorge D. 2017. Ameliorative effect of vitamin C against hepatotoxicity induced by emamectin benzoate in rats. *Human and Experimental Toxicology* **36**(7): 709–717.

Mousavi S, Escher U, Thunhorst E, Kittler S, Kehrenberg C, Bereswill S and Heimesaat M M. 2020. Ascorbic acid alleviates acute enteroclolitis in *Camphylobacter jejuni* infected mice. *Scientific Reports* **10**: 2921.

Rahal A and Kumar A. 2021. Strategies to combat antimicrobial resistance in Indian scenario. *The Indian Journal of Animal Sciences* **91**: 79–90.

Ramasamy T, Keerthana S, Srinivasan M R, Chandrasekar D, Porteen K, Borthakur A, Elamaran A and Sriram P. 2021. Molecular characterization of antibiotic resistance gene pattern of *Staphylococcus aureus* and *Escherichia coli* in mastitis affected dairy cows. *Indian Journal of Animal Research* **55**: 463–468.

Rosa G, Alba D F, Silva A D, Gris A, Mendes R E, Mostardeiro V B, Lopes T F, Schetinger M R C, Stefani L M, Lopes M T and Boiago M M. 2020. Impact of *Escherichia coli* infection in broiler breeder chicks: The effect of oxidative stress on weight gain. *Microbial Pathogenesis* **139**: 103861.

Saha O, Hoque M N, Islam O K, Rahaman M, Sultana M and Hossain M A. 2020. Multidrug-resistant avian pathogenic *Escherichia coli* strains and association of their virulence genes in Bangladesh. *Microorganisms* **8**: 1135.

Sarker M S, Mannan M S, Ali M Y, Bayzid M, Ahad A and Bupasha Z B. 2019. Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. *Journal of Advanced Veterinary and Animal Research* **6**: 272–277.

Shivaprasad D P, Taneja N K, Lakra A and Sachdev D. 2021. *In vitro* and *in situ* abrogation of biofilm formation in *E. coli* by Ascorbic acid through ROS generation, disruption of quorum sensing and exopolysaccharide production. *Food Chemistry* **341**: 128171.

Sriraam K S, Ramasamy T, Porteen K, Elamaran A, Pavithra M, Jagadeesh T and Ramesh S. 2022. Effect of atorvastatin and vitamin D against multi-drug resistant *Staphylococcus* spp and *Escherichia coli* isolated from bovine mastitis cases. *Indian Journal of Animal Research* DOI: 10.18805/IJAR.B-4969.

Surjagade S, Joshi B P, Mathakiya R A, Kapadiya B, Bansod A P, Jadhao A D, Prajapati R, Ghodasara D J, Dave C J and Jani P. 2020. Mortality pattern and pathological study of *E. coli* infection in broiler chickens. *Journal of Entomology and Zoology Studies* **8**: 867–872.

Topi D and Spahiu J. 2020. Presence of veterinary antibiotics in livestock manure in two Southeastern Europe countries, Albania and Kosovo. *Environmental Science and Pollution Research* **27**: 44552–44560.