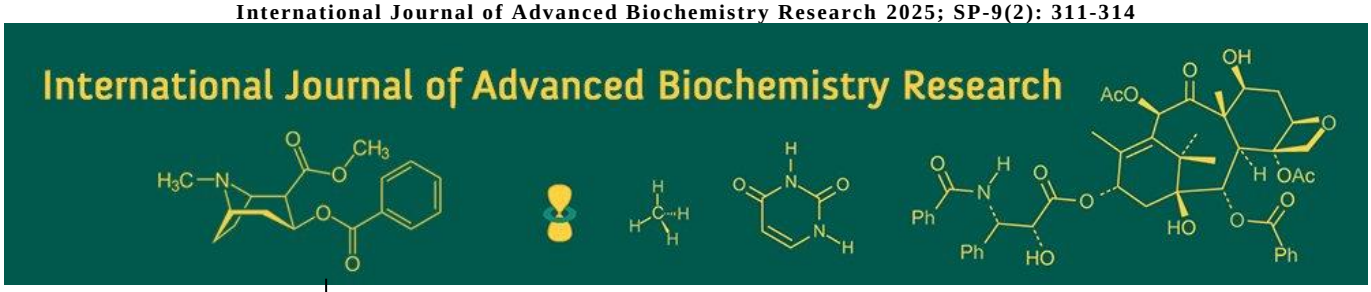

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Comparative efficacy of *Allium sativum* and levofloxacin on histopathological alterations in experimentally induced *Escherichia coli* infection in broilers

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Abstract
The present study evaluated the effect of *Allium sativum* bulb powder on induced *E. coli* infection in broiler birds by analyzing gross and histopathological alterations. A total of 150 Vencob broiler birds were divided into six groups (T_1-T_6) and subjected to different treatments. Birds in group T_1 were fed a plain diet (control), while those in group T_2 were administered *E. coli* broth (0.5 ml, 1x10⁹ CFU/ml) orally on the 8th day. Groups T_3, T_4, T_5, and T_6 received different combinations of *E. coli* broth, garlic powder (1.5 gm/kg of feed), and Levofloxacin (1gm/10 kg of body weight). Gross and histopathological changes were examined on the 7th, 14th, and 21st day post-infection. Severe gross pathological changes in group T_2 included tracheal congestion, lung edema, thickened air sacs, fibrinous pericarditis, perihepatitis, hemorrhagic kidneys, splenomegaly, and catarrhal exudation in the intestine. Birds in group T_4 exhibited milder gross lesions with focal lung consolidation and liver necrosis, while minimal changes were observed in groups T_5 and T_6. By the 21st day post-infection, no significant gross abnormalities were detected in any treatment groups except T_2. Histopathologically, birds in group T_2 showed marked catarrhal inflammation, desquamation of the tracheal mucosa, fibrinous bronchopneumonia, fibrinous airsacculitis, and pericarditis. Liver sections revealed fatty changes, focal coagulative necrosis, and fibroproliferative changes. Kidney sections showed hydropic degeneration, vacuolar changes, and proteinaceous casts, while spleen sections indicated lymphocyte depletion. Birds in group T_4 showed mild inflammatory changes, while minimal histoarchitectural alterations were noted in groups T_5 and T_6. The results demonstrated that garlic and its combination with Levofloxacin exhibited significant therapeutic potential by reducing gross and histopathological lesions in *E. coli*-infected broiler birds. The study highlights the antibacterial and protective effects of garlic, attributed to its high allicin content.

Keywords: *Allium sativum*, *Escherichia coli*, broiler and histopathology

Introduction
The poultry industry, primarily consisting of *Gallus gallus domesticus*, plays a crucial role in global food production, contributing significantly to the supply of meat and eggs. In India, the poultry sector is of particular importance, ranking third in egg production and sixth in chicken meat production (The Economic Survey 2021-22). This sector has experienced significant growth in recent years, with egg production increasing from 103.80 billion in 2018-19 to 138.38 billion in 2022-23. Similarly, poultry meat production rose from 4.06 million tonnes to 4.78 million tonnes during the same period. This growth underscores the increasing demand for poultry products, both domestically and internationally, highlighting the industry's significance in meeting global food security needs.

However, despite these advancements, the poultry industry faces persistent challenges, particularly those related to infectious diseases. Bacterial infections are a major concern as they lead to severe economic losses, resulting from increased mortality rates, reduced productivity, and high treatment costs. Among the bacterial diseases affecting poultry, avian colibacillosis caused by *Escherichia coli*) stands out as one of the most pressing issues. Avian pathogenic *E. coli* strains are responsible for various diseases such as respiratory tract infections, pericarditis, airsacculitis, and septicaemia, which contribute to high mortality

rates and the condemnation of carcasses (Amin *et al*., 2017; Panth, 2019) [3, 17]. Furthermore, the emergence of antimicrobial-resistant *E. coli* strains has compounded the difficulty of controlling these infections, posing a significant threat not only to animal health but also to public health through zoonotic transmission (Khan *et al.,* 2023) [12]. The misuse and overuse of antibiotics in poultry farming have exacerbated the problem of antimicrobial resistance, which has become a growing concern within the veterinary and public health sectors (Abd El-Tawab *et al*., 2016) [1].

The increasing prevalence of antibiotic-resistant bacteria has highlighted the need for alternative therapeutic strategies that can effectively address bacterial infections in poultry without contributing to the global antibiotic resistance crisis. In this context, the present study explores the potential of natural, plant-based alternatives with antimicrobial properties. One such promising alternative is garlic (*Allium sativum*), a widely used herb known for its rich bioactive composition and broad-spectrum antimicrobial activity. Garlic contains various bioactive compounds, including organosulfur compounds like allicin, which have demonstrated potent antibacterial effects against both Gram-positive and Gram-negative bacteria, including *E. coli* (Goncagul and Ayaz, 2010) [10]. Additionally, garlic has shown anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory effects, making it a viable candidate for enhancing poultry health and improving productivity (Nguyen *et al*., 2019; Melguizo-Rodríguez *et al.,* 2022) [16, 15].

Previous research has highlighted the potential benefits of incorporating medicinal plants like garlic into poultry feed. Garlic supplementation has been found to enhance growth performance, feed conversion efficiency, and immune response, while simultaneously reducing the prevalence of bacterial infections in poultry (Borgohain *et al*., 2019; Tesfaye, 2021) [4, 21]. Furthermore, garlic-based interventions have shown promising results in mitigating oxidative stress and improving the overall health of poultry by maintaining microbial balance in the gut, thereby contributing to better productivity and reduced disease incidence (He Ding *et al*., 2023) [11].

Levofloxacin, a broad-spectrum third-generation fluoroquinolone antibiotic, has been extensively used in veterinary medicine for the treatment of bacterial infections, including those caused by *E. coli*. Levofloxacin is highly effective against Gram-negative bacteria like *E. coli* and is particularly useful in treating systemic infections in poultry due to its high tissue penetration ability (Chen *et al.,* 2020). Despite its proven efficacy, concerns have arisen regarding the development of antimicrobial resistance in *E. coli* strains following the extensive and sometimes indiscriminate use of fluoroquinolones in poultry farming. The emergence of fluoroquinolone-resistant *E. coli* strains represents a significant threat to both animal and public health (Kumari *et al*., 2020) [13]. Regulatory restrictions on the use of antibiotics in poultry farming have further emphasized the need for alternative treatment options.

Given these challenges, a comparative approach involving conventional antibiotics, such as levofloxacin, and natural antimicrobial agents, such as garlic, has become an area of significant interest. While levofloxacin provides rapid therapeutic effects in controlling *E. coli* infections, plant-based alternatives like garlic have shown potential in offering antibacterial, immunomodulatory, and growth-promoting benefits without the adverse consequences associated with antibiotic resistance (Borgohain *et al*., 2019) [4]. This study aims to investigate the efficacy of garlic, specifically *Allium sativum* bulb powder, in controlling *E. coli* infections in broiler chickens by examining its effects on gross pathological and histopathological changes. The study also compares the effectiveness of garlic with levofloxacin, evaluating the potential of combining or replacing conventional antibiotics with herbal alternatives in poultry production.

The central hypothesis of this research is that garlic, due to its antimicrobial and antioxidant properties, can serve as a natural alternative to conventional antibiotics in reducing the morbidity and mortality associated with avian colibacillosis. Through controlled experimentation, this study seeks to provide scientific evidence supporting the use of garlic in poultry production as a sustainable solution to the growing issue of antibiotic resistance and bacterial disease outbreaks in the industry. By exploring the potential of plant-based alternatives like garlic, this research aims to contribute to the development of more sustainable and effective strategies for managing bacterial infections in poultry.

Materials and Methods

Experimental Design

The chicks in group-T_1 were given neither *E. coli* nor any treatment and kept as control group. Group-T_2 chicks were given broth of *E. coli* @ 0.5 ml ($1x10^9$ CFU/mL) by oral route at the age of 8th day. The chicks in group- T_3 were given powder of garlic bulbs @ 1.5 gm/ kg of feed by oral route through feed from 8th day till 15th day. The chicks in group-T_4 were given broth of *E. coli* @ 0.5 mL ($1x10^9$CFU/mL) by oral route at the age of 8th day and powder of garlic bulbs @ 1.5 gm/ kg of feed by oral route through feed from 8th day till 15th day. Whereas, chicks in group-T_5 were given broth of *E. coli* @ 0.5 mL($1x10^9$ CFU/mL) by oral route at the age of 8th day and Leofloxacin @ 1 gm/10 kg body weight for 7 days. While birds in group-T_6 were given broth of *E. coli* @ 0.5 mL($1x10^9$ CFU/mL) by oral route at the age of 8th day, powder of garlic bulbs @ 1.5 gm/kg of feed by oral route through feed from 8th day till 15th day and Leofloxacin @ 1 gm/10 kg body weight for 7 days.

IAEC Approval

The birds were reared according to standard management practices and the experimental protocol was approved from Institutional Animal Ethics Committee (IAEC) before start of experiment. All the birds in different groups were kept under close observation during whole experimental period.

Gross lesions

The gross pathological lesions in the visceral organs were recorded from dead and sacrificed birds at 7th day (15th day of age), 14th day (22nd day of age) and 21st day (29th day of age) of experiment.

Microscopic lesions

Pieces of suitable thickness of heart, lungs, trachea, liver, spleen, kidneys, intestine and air sacs were collected from the sacrificed birds of all the groups and those died due to infection and the samples were preserved in 10% neutral formal saline. The tissues were embedded in paraffin and

processed as per the standard procedure. The sections were cut at 4 to 5 µ thickness and were stained with Mayer's haematoxylin and eosin for microscopic examination (Culling, 1974) [7]. The detail microscopic findings in the tissue sections were recorded for each group at 7th day (15th day of age), 14th day (22nd day of age) and 21st day (29th day of age) of experiment.

Results

Clinical Symptoms

Chicks in Group T_1 (Healthy Control) and Group T_3 (Garlic Control) exhibited no abnormal clinical symptoms throughout the experimental period and maintained a healthy physiological state. Similarly, chicks in Group T_6 (*E. coli* infected, garlic, and levofloxacin treated) remained in good health without significant clinical symptoms for the duration of the trial. In contrast, chicks in Group T_2 (*E. coli* infected) displayed symptoms as early as 15 hours post-infection, including dullness, depression, ruffled feathers, weakness, huddling, anorexia, sneezing, nasal discharge, respiratory distress, and watery to semi-solid diarrhea with pasty vent. These symptoms are consistent with previous studies by Tandale (2019) [20], Rathod *et al.* (2021) [18, 19], Chavan *et al.* (2022) [5], and Enan *et al.* (2023) [9], corroborating the findings of the present study.

Chicks in Group T_4 (*E. coli* infected and garlic treated) showed minimal clinical symptoms. A few chicks exhibited dullness, depression, and reduced appetite, which lasted up to four days post-infection. Group T_5 (*E. coli* infected and levofloxacin treated) and Group T_6 experienced temporary dullness and depression lasting 12 to 24 hours post-infection, after which the birds appeared healthy for the remaining trial period. Observations of feed intake, body weight gain, and feed conversion ratio further supported these clinical findings.

Mortality

During the 29-day experimental period, an overall mortality rate of 2.67% was observed. In Group T_2, which was orally infected with *E. coli* culture, three chicks (12.33%) died. Mortality occurred during the first and second weeks of post-infection, with 4% occurring within the first week and 8.33% during the second week. Tandale *et al.* (2019) [20] reported a 16.66% mortality rate in *E. coli*-infected chicks, while Abeer *et al.* (2020) [2] observed a mortality rate of 40%, and Rathod *et al.* (2021) [18, 19] reported 14.25% mortality. These findings align with the current study's results. In Group T_4 (*E. coli* infected and garlic treated), one chick out of 25 died (4% mortality) during the first week post-infection. The results suggest that dietary garlic supplementation reduced mortality in broiler chicks. No mortality was observed in Groups T_5 (*E. coli* infected and levofloxacin treated) and T_6 (*E. coli* infected, garlic, and levofloxacin treated), indicating that the combination of levofloxacin in drinking water and garlic in feed had a protective effect against mortality. Tandale (2019) [20] also reported a protective effect of levofloxacin in *E. coli*-infected chicks, supporting the findings of the current study.

Gross Finding:

Gross pathological changes were observed to vary significantly among the experimental groups. Groups T_1 (Healthy control) and T_3 (Garlic control) did not exhibit any appreciable gross changes in visceral organs, including the trachea, lungs, air sacs, heart, liver, kidneys, spleen, and intestine, at any post-infection interval. In contrast, group T_2 (*E. coli* infected) displayed marked pathological changes, such as mild tracheal congestion at 7th and 14th days, severe pulmonary congestion, pneumonia, and necrosis, along with thickened, cloudy air sacs. The liver appeared pale with necrotic surfaces and fibrinous deposits, while the kidneys showed petechial hemorrhages and inflammation. Additionally, splenomegaly with petechial hemorrhages and hemorrhagic enteritis were prominent at earlier intervals. By the 21st day, these lesions had subsided, leaving residual changes such as mild congestion and focal necrosis in the liver and other organs. Treatment groups showed significant mitigation of these lesions. Birds in group T_4 (*E. coli* + Garlic) had mild lesions, such as tracheal congestion and focal liver necrosis, which resolved by the 21st day. Similarly, birds in group T_5 (*E. coli* + Levofloxacin) exhibited reduced lesion severity, with only mild congestion and pale liver observed throughout. Remarkably, birds in group T_6 (*E. coli* + Garlic + Levofloxacin) exhibited minimal or no gross pathological changes in all organs, maintaining near-normal gross anatomy.

Histopathological finding

Histopathological examinations confirmed significant lesions in *E. coli*-infected birds (group T_2). Tracheal sections revealed congestion, catarrhal inflammation, and mucosal desquamation until the 14th day, with residual infiltration by the 21st day. Pulmonary lesions included congestion, emphysema, pneumonic changes, fibrinous exudates, and thickened interalveolar septa, which persisted as mild changes at later intervals. Air sacs demonstrated fibrinous airsacculitis and congestion up to the 14th day, resolving by the 21st day. The heart exhibited focal necrosis, hemorrhages, fibrinous pericarditis, and inflammatory infiltration, which diminished over time. Liver sections showed focal necrosis, sinusoidal dilation, fatty degeneration, and inflammatory cell infiltration, while kidneys displayed severe degenerative changes, including hydropic and vacuolar degeneration, hyaline casts, and nephritic changes. The spleen revealed congestion, lymphocyte depopulation, and splenic artery thickening, whereas intestinal lesions involved villous blunting, goblet cell hyperplasia, and inflammatory infiltration. Birds treated with garlic (T_4) exhibited partial amelioration, with mild congestion and inflammation in trachea, lungs, liver, and kidneys. Birds treated with levofloxacin (T_5) exhibited fewer histopathological changes, such as occasional congestion and minor liver alterations. The combination treatment group (T_6) displayed the most significant improvement, with near-normal histoarchitecture in all organs. These findings underscored the synergistic and protective effect of combined garlic and levofloxacin treatment, consistent with previous studies (Deshmukh, 2006; Enan *et al.*, 2023; Kumari *et al.*, 2023) [8, 9, 14], and suggested its superior therapeutic efficacy in mitigating *E. coli*-induced damage. These findings suggest the potential therapeutic efficacy of garlic and levofloxacin, particularly in combination, in alleviating gross and histopathological lesions caused by *E. coli* infections in broiler birds.

Conclusion

Present study concluded that garlic (Allium sativum) and levofloxacin individually exhibit therapeutic efficacy in

mitigating *E. coli*-induced damage in broiler chickens. Garlic supplementation effectively reduces mortality, clinical symptoms, and histopathological lesions while enhancing overall health. Levofloxacin demonstrates rapid symptom control and significant lesion mitigation. The combination of garlic and levofloxacin provides the most significant improvement, with near-normal gross and histological findings, underscoring their synergistic effect. These results highlight garlic as a viable natural alternative to antibiotics, offering sustainable and effective strategies for managing bacterial infections in poultry while addressing the growing concern of antimicrobial resistance.

Conflict of Interest
None.

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