



Assessment of Herbal Substitutes for Antibiotics: Impact of Curcumin, Green Tea, and Garlic Extracts on Growth, Liver Health, and Oxidative Stress in *E. Coli*-Infected Chickens



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Abstract

AVIAN colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC) serotype O78, poses a major threat to poultry production through high morbidity, mortality, and economic losses. While antibiotics such as ciprofloxacin remain the mainstay of treatment, increasing antimicrobial resistance necessitates evaluation of alternative therapies. This study aimed to compare the antibacterial, antioxidant, and histopathological effects of curcumin, garlic, green tea and some combinations against experimental *E. coli* O78 infection in broiler chickens, with ciprofloxacin used as a reference standard. A total of 240 chicks were randomly allocated into eight groups, including negative, positive controls, curcumin, green tea, garlic, ciprofloxacin, and combination regimens. Birds (except negative control) were orally challenged with *E. coli* O78 at day 14. Clinical signs, mortality, growth performance, lesion scores, histopathological changes, and antioxidant markers (CAT, SOD) were assessed. The positive control group showed severe colibacillosis with marked hepatic damage, while all phytochemical treatments produced clear clinical and histopathological improvement. Curcumin, green tea, and garlic reduced lesion severity by Day 19 and showed further healing by Day 25, with curcumin + green tea offering notable early protection. Ciprofloxacin combination with phytochemicals at half dose achieved the greatest lesion resolution. Antioxidant results showed higher CAT and SOD activities in curcumin-based treatments, while ciprofloxacin and infected controls had the lowest values. Histopathological examination revealed that birds treated with the triple combination (G8) and dual-phytochemicals (G5) exhibited significantly improved liver architecture with minimal inflammatory infiltration compared to the severe hepatic damage observed in the positive control (G2) and enhanced antioxidant status in *E. coli*-infected birds so it can enhance or complement conventional antibiotic therapy.

Keywords: *Escherichia coli* O78, broiler chickens, Herbal Extracts, antimicrobial resistance, oxidative stress, curcumin.

Introduction

Avian colibacillosis is caused by avian pathogenic *Escherichia coli* (APEC), with serotype O78 being among the most prevalent and virulent strains in poultry. It is a complex disease that leads to high morbidity, fluctuating mortality, reduced feed conversion, carcass condemnation, and substantial economic losses in poultry production worldwide [18]. APEC strains can colonize the intestinal mucosa and disseminate to extra-intestinal organs, producing systemic infections that compromise bird

health and performance. Antibiotics remain the cornerstone for preventing and treating colibacillosis [6], yet their continuous and indiscriminate use has contributed to the alarming rise of multidrug-resistant (MDR) *E. coli* strains [14]. This not only reduces therapeutic efficacy but also poses public health risks through zoonotic transmission of resistance genes. Moreover, long-term use of antibiotics increases the risk of adverse drug effects, necessitating the exploration of alternative or adjunct strategies. [26].

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Phytochemical compounds, including garlic (*Allium sativum*), green tea (*Camellia sinensis*), and curcumin, have demonstrated promising antimicrobial and immunomodulatory effects in poultry [3, 22]. Garlic has been reported to enhance phagocytic activity and accelerate clearance of MDR *E. coli* O78 in broilers [3]. Green tea extract exhibits antimicrobial, anti-inflammatory, and gut-protective roles, though its direct efficacy against colibacillosis remains less established [23].

Among these phytochemicals, curcumin stands out as a dual-action candidate. It is a hydrophobic polyphenolic compound derived from turmeric (*Curcuma longa*), widely used as a spice and traditional remedy. Curcumin possesses potent anti-inflammatory, antioxidant, and antibacterial properties [15, 9]. Mechanistically, curcumin's efficacy is largely attributed to its ability to disrupt bacterial cell division by targeting the FtsZ protein (Filamenting temperature-sensitive mutant Z) is a critical prokaryotic protein that acts as the primary orchestrator of bacterial cell division) which considered a key prokaryotic cytoskeletal element. Our findings align with molecular docking models suggesting that curcumin binds to the GTPase (specialized enzyme releasing the energy required to regulate bacterial cell division) catalytic pocket of FtsZ. This binding inhibits the GTPase enzymatic activity required for Z-ring assembly, thereby halting cytokinesis and suppressing *E. coli* proliferation [12]. It further interferes with DNA replication, reduces biofilm formation, and impairs bacterial adhesion and motility. In parallel, curcumin boosts host defences by increasing antioxidant enzymes such as glutathione peroxidase and attenuating reactive oxygen species (ROS), thereby protecting tissues from oxidative stress [10].

The overall purpose of this research is to assess the therapeutic value of three natural compounds—curcumin, green tea, and garlic—when used alone or in selected combinations against experimental *Escherichia coli* O78 infection in broiler chickens.

In order to demonstrate the potential of these natural chemicals as supportive agents in addition to traditional antibiotic treatments, the study attempts to determine the best successful single treatment or combination, especially the triple regimen.

Material and Methods

Experimental birds and Housing

A total of 245-day-old, unsexed Cobb 500 broiler chicks were acquired from a commercial hatchery. Upon arrival, birds were randomly assigned to eight experimental groups (3 replicates) to floor pens (1.5 x 1.5 m), each pen had 10 birds furnished with clean wood-shavings bedding in a climate-controlled poultry facility. Each pen was equipped with nipple drinkers supplying fresh water and feeders

dispensing a typical broiler ration ad libitum. A continuous lighting program (24 hours of light) was implemented during the initial week, succeeded by 23 hours of light and 1 hour of darkness for the duration of the experimental phase. The trial lasted 28 days.

Experimental Design and Grouping

Before grouping, 5 chicks were sacrificed and examined for bacteriological examination to make sure there was no *E. coli* infection then 245 chicks were randomly assigned to eight experimental groups, with 30 birds per group. Each group was further divided into three replicates of 10 chicks. The groups were arranged as (table 1)

Natural Compounds and Antibiotic

Curcumin: curcumin powder [95%] curcuminoids was incorporated as 200 mg/L in drinking water [10]

Green Tea: Green tea extract standardized to 50% Epigallocatechin gallate (EGCG) and 90% total polyphenols. was dissolved in drinking water at a concentration of 500 mg/L drinking water [22]

Green tea extract used was prepared via aqueous-ethanolic extraction to ensure maximum recovery of bioactive compounds. The total polyphenol content (TPC) was quantitatively estimated using the Folin-Ciocalteu colorimetric method, as previously described [25]

Garlic: Dried powder standardized to 1.3% allicin content was mixed with water at 500 mg/L [24]

To ensure batch consistency and reproducibility, all supplements were obtained from a single manufacturing lot and all extracts were stored in airtight, light-protected containers at 4°C to maintain bioactive stability throughout the trial

The antibiotic: Ciprofloxacin 20% was purchased from Avico® pharmaceutical company and administered via drinking water at a therapeutic dose of 1 ml/L for three consecutive days (Days 15–17) "starting 24 h after infection" All natural treatments (G3- G8) were initiated on Day 1 and continued until the end of the trial.

Experimental Infection

Strain Preparation

A virulent field isolate of *Escherichia coli* O78 (avian pathogenic *E. coli*, APEC) was kindly provided from Microbiology Department, Animal Health Research Institute, Agriculture Research Center, Egypt. The organism was grown in Luria-Bertani (LB) broth at 37°C for 18 h to reach approximately 1×10^8 CFU/mL, confirmed by serial dilution and plating.

Inoculation Procedure

At 14 days of age, birds in Groups G2–G8 were challenged orally with 1 mL of the prepared inoculum, delivering 1×10^8 CFU per [2]. Birds in the negative control group (G1) received 1 mL of sterile water. All groups were monitored daily for clinical signs and mortality.

Growth Performance

Body weights were recorded at 14 (time of infection) and 25 days of age (end of experimental phase). Average body weight gain (BWG) was calculated per replicate. Feed intake was recorded, and feed conversion ratio (FCR) was determined according to the criteria described [7].

Necropsy and Lesion Scoring

Sampling for gross lesion evaluation was performed at two specified time points: the First Lesion Scoring was 5 days post infection (DPI) and the Second Lesion Scoring was 11 DPI. At each time point, a sub-sample of three birds per group were slaughtered for post-mortem examination.

Serum Biochemistry

Serum samples were analyzed for antioxidant biomarkers, including catalase (CAT) and superoxide dismutase (SOD) at 0,5 DPI and 11 DPI to assess oxidative stress status [16].

Histopathological Examination

Liver and intestine tissues (three per group) were taken 5 DPI and 11 DPI then preserved in 20% neutral-buffered formalin, embedded in paraffin, and sectioned at 3 μ m. Hematoxylin and eosin (H&E) staining was conducted in accordance with established histological techniques [8], and the slides were analyzed microscopically. The severity of histopathological alterations in the liver was quantitatively assessed using a standardized scoring system on a scale of 0 to 3, as previously described by [27]. The scoring criteria were defined as follows: 0 indicated the absence of lesions, 1 represented slight lesions, 2 denoted moderate lesions, and 3 signified severe lesions

Statistical analysis

All data were analyzed as a completely randomized design by one-way ANOVA using the statistical application SPSS version 20.0 for Windows (SPSS, Inc., Chicago, IL, USA). Duncan's post hoc test ($p < 0.05$) was conducted to determine the significance level between the experimental groups.

Results

Clinical Signs and Mortality

Chicks in the Negative control group [NC-G1] remained healthy with no observed clinical abnormalities recording 0% mortality throughout the trial. In contrast, the Positive control group [PC-G2]

developed typical colibacillosis signs such as depression, ruffled feathers, diarrhea, and respiratory distress within 2–3 days post-challenge. Mild clinical signs were also recorded in groups treated with phyto-genic additives (Curcumin [CUR-G3], Green tea [GT-G4], Garlic [G-G7], whereas Ciprofloxacin [CIP-G6] and the combined regimen G5 (Curcumin + Green tea) and [Curcumin + Green tea + Ciprofloxacin- G8] showed the most effective clinical protection with no observed mortality. (Table 2).

Gross pathology scoring of liver

On day 19, the untreated infected group exhibited significant liver damage, characterized by pronounced perihepatitis and congestion. The Garlic (G7) and Curcumin (G3) groups demonstrated decreased hepatic lesion severity relative to the positive control. The CUR+GT (G5), which got only Curcumin and Green Tea at this time, had a significant reduction in liver lesions, demonstrating a robust preemptive hepatoprotective and anti-inflammatory impact. By Day 25, the combined group (CUR+GT), the garlic group also the curcumin group exhibited nearly total clearance of liver lesions. The CUR+GT+half-CIP group (G8) had significant hepatic recovery, affirming that early herbal medication facilitated subsequent liver healing. (figure 1 and figure 2).

Growth performance

At Day 14, no significant differences ($p > 0.05$) were observed in TBW and TFI between the Positive Control and the treatment groups. However, at Day 25 The Positive Control (969.34 ± 68.26) exhibited the lowest TBW and a significant reduction ($p < 0.05$) in TFI. Prior to the *E. coli* O78 infection (Day 14), no significant differences ($p > 0.05$) were observed in the Feed Conversion Ratio (FCR) among the groups. However, Day 25 revealed that phyto-genic treatments yielded the highest feed conversion efficiency, notably Curcumin (1.32 ± 0.04), Garlic (1.32 ± 0.02), and the combination of Curcumin + Green Tea (1.35 ± 0.02). The three groups demonstrated significant superiority over both the control groups and the antibiotic group ($p < 0.05$). In contrast, the Ciprofloxacin group had the lowest feed conversion efficiency (1.62 ± 0.05), with its feed conversion ratio substantially exceeding that of the Curcumin, Garlic, and Curcumin + Green Tea groups ($p < 0.05$). The Control + group (1.57 ± 0.10) and the Control - group (1.56 ± 0.06) exhibited statistically inferior efficiency compared to the three highest-performing extract groups ($p < 0.05$). The notable enhancement in FCR occurred despite the Curcumin (1352.05 ± 8.67) and Garlic (1592.90 ± 39.82) groups exhibiting reduced Total Feed Intake (TFI) compared to the Ciprofloxacin group. Although the Total Weight Gain study revealed no statistically significant differences among the groups ($p > 0.05$), the FCR data unequivocally demonstrate

that the phytogetic treatments significantly improved nutrient consumption and yielded a more economically advantageous outcome compared to the antibiotic standard. (Table 3)

Biochemistry assay

Catalase activity (CAT) at day zero (before infection) as shown in table 2, Groups receiving Curcumin (G3) and the Curcumin + Green Tea combination (G5) exhibited the highest CAT activity (0.58 ± 0.01 and 0.55 ± 0.03), respectively), showing a statistically significant ($p < 0.05$) increase compared to all other groups while, there was no significant difference ($p > 0.05$) between the Negative Control (G1), Positive Control (G2), and the Ciprofloxacin group. Five days post-infection, CAT activity exhibited a notable pattern of significant variance between treatments ($p < 0.05$). The Garlic (1.18 ± 0.04) group demonstrated the highest CAT activity, whereas the Curcumin, Curcumin + Green, and Curcumin + Green Tea + half-Cipro groups displayed increased CAT activity, with no significant differences observed among them. Conversely, markedly reduced values were noted in the ciprofloxacin (0.483 ± 0.076) group. By 11 days post-infection, CAT activity markedly decreased in all groups ($p < 0.05$). The minimum values were noted in the Control + (0.35 ± 0.03) and ciprofloxacin groups, whereas the maximum CAT activity during the resolution phase was detected in the Curcumin (0.88 ± 0.02) and Curcumin + Green Tea groups. (table 4)

Superoxide Dismutase (SOD) activity

Throughout all sampling intervals (day 0, day 5, and day 11), SOD activity exhibited numerical variance among the experimental groups; however, these discrepancies were not statistically significant ($p > 0.05$) as per Duncan's multiple range test (Table 3). The lowest SOD values were consistently recorded in the infected and ciprofloxacin groups. The peak SOD activity at all time intervals was consistently observed in the curcumin-based treatments, specifically Curcumin alone and Curcumin + Green Tea, which exhibited the most substantial numerical increases in SOD levels during the experimental duration, notwithstanding the absence of statistical significance ($p > 0.05$). (Table 5)

Histopathological Findings of liver

Different experimental groups' liver tissues were subjected to histopathological investigation, which revealed apparent variations in microscopic lesions. In the NC group at 19 days (Fig. 3 A) and 25 days (Fig. 3 E) sacrifices, the liver tissues showed no pathological alterations and preserved structural integrity. Hepatic lobules, central veins, blood sinusoids and hepatocytes were all well-organized, with hepatocytes displaying clear cytoplasm and distinct nuclei and no substantial inflammatory

response. Conversely, the PC group showed extensive liver damage at day 19 than at day 25 (Fig. 3 B-D and F-H). This group exhibited pronounced hepatocellular degeneration, cell swelling, substantial inflammatory cell infiltration and large areas of bridging necrosis. Hepatocytes adjacent to necrotic zones revealed considerable swelling and vacuolation, with these areas densely infiltrated by inflammatory cells forming massive nodular aggregates, particularly apparent at the second sacrifice. Furthermore, portal triad in the E. coli group showed vascular congestion, fibrous connective tissue proliferation and biliary hyperplasia.

Single-agent therapies utilizing curcumin(G3) or green tea(G4) provided moderate protective effects, with liver lesions including lymphocytic cell aggregation and hepatocellular degeneration were mild at 19 days (Fig. 4 A & B) milder at 25 days (Fig. 4 G & H) sacrifices. Meanwhile, the curcumin and green tea combination without ciprofloxacin(G5) demonstrated mild congestion of hepatic sinusoids with less severe lymphocytic aggregation (Fig. 4 C & I). Similarly, the combination therapy consisting of curcumin, green tea, and half-ciprofloxacin(G8) maintained a well-preserved liver structure with only mild vacuolation and minimal inflammation (Fig. 4 D & J). In contrast to the E. coli group(G2), the garlic treatment(G7) (Fig. 4 E & K) and the ciprofloxacin monotherapy(G6) (Fig. 4 F & L) demonstrated organ-specific improvements, with liver architecture largely preserved and less severe lymphocytic cell aggregation.

Histopathological (Microscopical) Scoring Description of Liver Lesions

The untreated E. coli-infected group(G2), liver lesions demonstrated a reduction from near maximal severity (mean score of 3) at 19 days post-infection (dpi) to severe (mean score of 2.8) by 24 dpi. This was marked by bridging necrosis, panlobular inflammatory infiltration, and hepatocellular degradation. The combination therapy of curcumin, green tea, and half-ciprofloxacin(G8) demonstrated significant hepatoprotection, as indicated by a decrease in liver lesions from moderate (average score of 1.0) at 19 dpi to modest (average score of 0.6) by 25 dpi. The combination of curcumin and green tea, excluding ciprofloxacin(G5), shown significant efficacy, as liver lesions diminished from moderate (mean score of 1.0) to mild (mean score of 0.8). Significantly, garlic treatment (G7) demonstrated organ-specific advantages, with liver lesions improving from moderate (mean score of 1.8) to mild-moderate (mean score of 1.2). Monotherapy employing curcumin(G3) or green tea (G4) exhibited moderate protective effects, as liver lesions diminished from moderate (average scores of 2.0 to 2.2) to mild (average scores of 1.4 to 1.6). Ciprofloxacin monotherapy (G6) produced

intermediate outcomes, with liver lesions decreasing from moderate (mean score of 1.6) to mild-moderate (mean score of 1.2). In all experimental groups, liver lesions consistently scored between 0.1 and 0.3 points, indicating the liver's heightened susceptibility to necrotic damage and its comparatively diminished regenerating capabilities. All treatment groups revealed a progressive clearance of lesions from 19 to 25 dpi, while the untreated control group showed a deterioration in pathological conditions.

Discussion

Colibacillosis is a major contributor to morbidity and mortality in chickens, resulting in considerable economic losses within the poultry sector. The unregulated application of antibiotics against poultry infections has led to the development of antimicrobial resistance in *E. coli*, resulting in treatment failures. The rising prevalence of antibiotic-resistant *E. coli* strains challenges treatment efforts, necessitating the investigation of new therapeutic methods [13]. This experimental layout allows for a comprehensive evaluation in three main areas. First, growth performance is monitored by recording body weight, weight gain, and feed conversion ratio to determine the ability of each treatment to counteract the growth-depressing effects of infection. second, antioxidant status is assessed by measuring the activities of catalase (CAT) and superoxide dismutase (SOD), which serve as indicators of the degree to which the treatments reduce infection-induced oxidative stress. third, histopathological evaluation of liver to see to what extent did the extracts protect against infection. The study assessed the therapeutic efficacy of natural phytochemical extracts (Curcumin [CUR], Green tea [GT], Garlic[G]) and some combinations of curcumin and green tea [G5] with or without ciprofloxacin relative to Ciprofloxacin alone [CIP] in managing an *E. coli* O78 infection. A key finding of this study is the combination of the curcumin+ green tea with half dose of ciprofloxacin, which utilized only a 50% therapeutic dose of Ciprofloxacin. The experimental *E. coli* challenge effectively elicited typical manifestations of colibacillosis, resulting in significant declines in growth performance, oxidative stress dysregulation, and pronounced hepatic damage in the untreated positive control group. This aligns with prior studies detailing systemic inflammation, hepatocellular degeneration, and diminished productivity in broilers subjected to *Escherichia coli* infection [19]. In contrast, broilers administered phytochemical additives (Curcumin [CUR], Green tea [GT], Garlic[G]) displayed only mild clinical symptoms and a significant enhancement in performance metrics. The reduction in mortality in CUR+GT and CUR+GT+half-CIP is rooted in the multi-targeted antimicrobial activity of the extracts. Curcumin effectively inhibits *E. coli* growth by targeting the FtsZ protein, essential for bacterial cell

division as reported by [12]. The enhanced Feed Conversion Ratio (FCR) noted in the curcumin, garlic and curcumin+green tea groups was significantly better than both the PC and full-dose Ciprofloxacin groups. These findings align with [1], who noted that garlic supplements augment nutrient digestibility and optimize metabolic efficiency. In gross pathology, The *E. coli* challenge caused severe oxidative stress, as evidenced by the high liver lesion scores in the PC group compared to mild and moderate scores in phytochemical groups. That came with marked increase in catalase activity among phytochemical-treated groups, especially in those receiving garlic, curcumin, and a combination of curcumin and green tea peaked during the acute phase (Day 5 post-infection/Day 19 of age) as the birds' immune systems responded to the challenge. By Day 25, a general decline in enzyme activity was noted in all groups as the systemic inflammation began to resolve, though the phytochemical-treated groups maintained significantly higher levels compared to the Positive Control (G2) which substantiates the potent antioxidant properties of these compounds. This observation aligns with previous research indicating that allicin and polyphenols bolster endogenous antioxidant enzyme systems, including catalase (CAT), superoxide dismutase (SOD), thereby mitigating oxidative damage in hepatic and intestinal tissues by [20] and aligns with the finding of [17] who reported that curcumin and combination with selenium increased the total antioxidant capacity and superoxide dismutase (SOD) activity, which indicates that these compounds effectively triggered the host's primary defense, consistent with findings that curcumin improves antioxidant status in broilers under physiological stress [16].

Additionally, it corroborates findings by [9] which reported that curcumin at 200 mg/l enhanced catalase activity in poultry. Despite the lack of statistical significance in SOD activity differences, the consistently elevated numerical values in curcumin-based groups align with prior research suggesting that curcumin augments superoxide scavenging capacity, stabilizes cellular membranes, and mitigates free radical-induced cellular damage under physiological stress conditions as mentioned by [28] This statistical significance like phenomenon may be attributed to high biological variability inherent in avian antioxidant responses. Histopathological assessment corroborated the biochemical and clinical observations, as severe hepatocellular degeneration, inflammatory infiltration, vascular congestion, and bridging necrosis identified in the untreated infected group align with the established pathological characteristics of avian colibacillosis documented in the literature by [21]. In contrast, the significant preservation of hepatic architecture and diminished inflammatory cell aggregation in the phytochemical and combined

treatment groups robustly affirm the hepatoprotective properties of these natural compounds. curcumin and garlic exhibit comparable hepatoprotective effects in experimental models this is likely due to the ability of polyphenols and curcuminoids to reduce inflammatory cell infiltration and maintaining liver structural integrity as previously discussed by [20,28]. The combination of curcumin and green tea, both independently and in conjunction with ciprofloxacin, yielded the most significant diminution in microscopic lesion scores revealing that liver damage was near maximal (mean score of 3) at Day 19 in the PC group (G2), with only slight natural improvement by Day 25 (mean score 2.8). However, Group G8 demonstrated a significant healing trend, with lesion scores decreasing from 1.0 (moderate) to 0.4–0.6 (slight) over the same period which indicates a synergistic interaction between polyphenols and curcuminoids. This phenomenon illustrates augmented antimicrobial, anti-inflammatory, and antioxidant effects when phytochemicals are utilized collectively rather than in isolation. The performance of the Curcumin + Green Tea group, which was nearly equivalent to and, in certain parameters, superior to ciprofloxacin alone, underscores the increasing evidence that phytochemicals may function as viable alternatives or supplements to conventional antibiotics in poultry production, especially amid escalating antimicrobial resistance [25]. While Ciprofloxacin monotherapy effectively controlled mortality, its significantly reduced efficacy regarding feed efficiency and antioxidant restoration suggests a metabolic strain or microbiota disturbance linked to extended full-dose antibiotic use which corroborates with [4]. The success of the half-dose combination reinforces the global trend toward integrating natural, plant-derived additives to reduce the chemical burden in poultry production.

Conclusion

The experimental groups were strategically designed to evaluate the antibiotic-sparing potential of phytochemical extracts. While the Negative Control (G1) and Positive Control (G2) validated the infection model, the monotherapy groups (G3, G4, and G7) provided a baseline for individual herbal efficacy. The most critical finding lies in Group G8, where the combination of Curcumin and Green Tea allowed for a 50% reduction in the therapeutic dose of Ciprofloxacin while maintaining superior outcomes in mortality, liver health, and feed efficiency compared to full-dose monotherapy (G6).

This study definitively demonstrates that curcumin, green tea, and garlic, particularly in combinations, substantially reduce *E. coli*-induced pathological damage and death in broiler chickens. By augmenting antioxidant defense mechanisms (CAT and SOD), preserving liver structural integrity, and promoting growth performance, these phytochemicals offer powerful and promising adjunct strategies. These findings provide robust experimental evidence for the utilization of natural extracts as synergistic supplements to address antibiotic resistance and enhance sustainable poultry health management.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All experimental procedures were conducted in accordance with the guidelines of the Animal Welfare Committee, Faculty of Veterinary Medicine, Benha University, under protocol number BUFVTM 02-09-24

TABLE 1. group numbers, names and specialized treatments

Group No	Group (n=30)	Replicates (n=3)	Infection Status (Day 14)	Treatment (Dosage/Route/Duration)
1	NC		Negative (water)	None (Negative Control)
2	PC		infected (1×10^8 CFU)	None (Positive Control)
3	CUR	10 chicks/rep	infected	Curcumin (200 mg/L DW)
4	GT		infected	Green Tea (500 mg/L DW)
5	GT+CUR		infected	Green Tea + Curcumin (full dose)
6	CIP		infected	Ciprofloxacin (full dose-1ml/L, DW, D15-17)
7	G		infected	Garlic (500mg/L DW)
8	GT+CUR+CIP		infected	Curcumin + Green Tea (Full doses) + Ciprofloxacin (half dose-0.5ml/L)

*NC (Negative Control): Non-infected birds receiving sterile water; PC (Positive Control): Birds infected with *E. coli* (1×10^8 CFU) on Day 14 with no treatment; CUR: Curcumin (200 mg/L in drinking water); GT: Green Tea (500 mg/L in drinking water); GT+CUR: Green Tea + Curcumin (full dose); CIP: Ciprofloxacin (1 mL/L in drinking water, administered Days 15–17); G: Garlic (500 mg/L); GT+CUR+CIP: Combined treatment with full doses of Green Tea and Curcumin plus a half-dose of Ciprofloxacin (0.5 ml/L). DW: Drinking Water; CFU: Colony Forming.

TABLE 2. Number and percent of mortalities in every each treatment.

Group No	Group	Total	Mortality No	Mortality percent (%)
1	NC	30	0	0
2	PC	30	6	20
3	CUR	30	1	3.3
4	GT	30	3	10
5	GT+CUR	30	0	0
6	CIP	30	2	6.6
7	G	30	1	3.3
8	GT+CUR+CIP	30	0	0

*NC (Negative Control): Non-infected birds; PC (Positive Control); CUR: Curcumin; GT: Green Tea ; GT+CUR: Green Tea + Curcumin; CIP: Ciprofloxacin; G: Garlic; GT+CUR+CIP: Combined treatment of Green Tea and Curcumin plus a half-dose of Ciprofloxacin.

TABLE 3. Effect of curcumin, green tea, garlic and their combination on growth performance of E. coli challenged chicks.

	PC	CUR	GT	CUR+GT	CUR+GT +CIP	G	CIP	NC
AFI14	638.76± 16.53 ^a	487± 3.56904 ^a	579.05± 18.57 ^d	555.38± 51.14 ^{b,c}	550.05± 11.87 ^{b,c}	484.13± 6.92 ^a	643.13± 13.91 ^a	608.95± 27.21 ^{ab}
AFI25	1051.71± 30.50 ^b	894.53± 59.63 ^{c,d}	929.24± 35.16 ^{b,c,d}	1048.58± 13.21 ^b	941.19± 9.06 ^{b,c,d}	840.43± 54.18 ^{bc}	992.75± 49.09 ^{bc}	1183.10± 13.53 ^a
BW14	402.67± 33.93 ^b	450.33± 4.91 ^{ab}	425± 15.31 ^b	427.67± 3.67 ^b	407.33± 14.90 ^b	489.33± 20.99 ^a	424.44± 7.22 ^b	427.33± 5.49 ^b
BW25	836± 14.43 ^d	1083.67± 30.96 ^a	1069± 37.43 ^a	1012.33± 12.45 ^{ab}	1005.67± 41.35 ^{ab}	1081.33± 25.98 ^{bc}	976.67± 10.65 ^d	913.67± 14.53 ^{c,d}
WG14	362.67± 33.93 ^b	410.33± 4.91 ^{ab}	385± 15.31 ^{ab}	388.33 ± 3.38 ^{ab}	367.33 ± 14.90 ^{ab}	416.00 ± 14.00 ^a	387.00 ± 2.52 ^{ab}	395.00 ± 9.29 ^{ab}
WG25	566.33 ± 34.62 ^{ab}	663.67 ± 12.73 ^a	589.58 ± 48.99 ^{ab}	644.33 ± 22.19 ^a	598.33 ± 26.54 ^{ab}	658.67 ± 8.37 ^a	532.00 ± 18.00 ^b	578.67 ± 38.26 ^{ab}
FCR14	1.61 ± 0.04 ^a	1.44 ± 0.03 ^c	1.44 ± 0.03 ^b	1.38 ± 0.02 ^b	1.57 ± 0.06 ^a	1.38 ± 0.06 ^{ab}	1.48 ± 0.09 ^{ab}	1.46 ± 0.06 ^{ab}
FCR25	1.58 ± 0.03 ^a	1.25 ± 0.03 ^b	1.45 ± 0.06 ^{ab}	1.26 ± 0.09 ^b	1.68 ± 0.18 ^a	1.26 ± 0.07 ^b	1.69 ± 0.05 ^a	1.45 ± 0.05 ^{ab}
TFI	1449.90 ± 108.36 ^a	1352.05± 8.67 ^a	1508.28 ± 25.89 ^a	1420.66 ± 19.95 ^a	1583.48 ± 166.44 ^a	1592.90 ± 39.82 ^a	1476.89 ± 43.49 ^a	1390.47 ± 52.72 ^a
TBW	969.34 ± 68.26 ^a	1065.86 ± 27.86 ^a	1069.15 ± 37.32 ^a	959.14 ± 21.71 ^a	1005.71 ± 41.17 ^a	952.43 ± 0.00 ^a	952.43 ± 0.00 ^a	1013.72 ± 43.32 ^a
TWG	929.34 ± 68.26 ^a	1025.86 ± 27.86 ^a	1029.15 ± 37.32 ^a	919.14 ± 21.71 ^a	965.71 ± 41.17 ^a	912.43 ± 0.00 ^a	912.43 ± 0.00 ^a	973.72 ± 43.32 ^a
TFCR	1.57 ± 0.10 ^a	1.32 ± 0.04 ^b	1.43 ± 0.03 ^{ab}	1.35 ± 0.02 ^b	1.53 ± 0.08 ^a	1.32 ± 0.02 ^b	1.62 ± 0.05 ^a	1.56 ± 0.06 ^a

* Means with different superscripts (a, b, c, and d) within a column are profoundly different at $p < 0.05$ within the same row. Values are given as the mean \pm SE. *NC: non-treated non-challenged group; PC: Challenged with E. coli O78; CUR: treated with curcumin in drinking water (200mg/L) from day one to of the trial and challenged with E. coli O78; GT: Treated with green tea in drinking water 500 mg/L from day one of the trial and challenged with E. coli O78; CUR+GT: Treated with both curcumin and green tea and challenged with E.coli; CIP:challenged with E.coli and treated with ciprofloxacin for 3 days post challenge; G:treated with garlic extract (500mg/L) from day one to of the trial and challenged with E. coli O78. AFI: Average Feed Intake (g/chick); BW: Body Weight (g/chick); WG: Weight Gain (g/chick); FCR: Feed Conversion Ratio.

TABLE 4. Effect of curcumin, green tea, garlic and combinations on catalase enzyme level of *E. coli* challenged chicks.

Group No	Group	Day 0 (Pre-Infection)	Day 5 (Peak Infection)	Day 11 (Resolution)
1	NC	0.28±0.01 ^c	0.55±0.031 ^b	0.45±0.03 ^c
2	PC	0.25±0.03 ^c	0.5±0.03 ^b	0.35±0.03 ^c
3	CUR	0.58±0.01 ^a	1.12±0.02 ^a	0.88±0.02 ^a
4	GT	0.32±0.04 ^{b,c}	0.54±0.05 ^b	0.47±0.04 ^c
5	CUR +GT	0.55±0.03 ^a	1.13±0.02 ^a	0.85±0.03 ^a
6	CIP	0.3±0.03 ^c	0.48±0.04 ^b	0.38±0.04 ^c
7	G	0.4±0.03 ^b	1.18±0.04 ^a	0.7±0.06 ^b
8	CUR+GT+CIP	0.4±0.03 ^b	1.1±0.06 ^b	0.72±0.04 ^b

*Duncan's test represents differences between different groups at probability $p < 0.05$. Means with different superscripts (a, b, c, and d) within a column are profoundly different at $p < 0.05$. Values are given as the mean \pm SE .NC: non-treated non-challenged group; PC: Challenged with *E. coli* O78; CUR: Challenged with *E. coli* O78 and treated with curcumin in drinking water (200mg/L) from day one to of the trial; GT: challenged with *E. coli* O78 and treated with green tea in drinking water 500 mg/L from day one of the trial;CUR+GT:Challenged with *E.coli* and treated with both curcumin and green tea;CIP:challenged with *E.coli* and treated with ciprofloxacin for 3 days post challenge;G:Garlic extract (500mg/L) from day one to of the trial.

TABLE 5. Effect of curcumin, green tea, garlic and their combination on superoxide dismutase enzyme level of *E. coli* challenged chicks.

Group No	Group	Day 0 (Pre-Infection)	Day 5 (Peak Infection)	Day 11 (Resolution)
1	NC	10.33±0.88 ^a	23±1.73 ^a	16.67±1.45 ^a
2	PC	11±0.58 ^a	22±1.15 ^a	16.33±0.88 ^a
3	CUR	32.53±2.79 ^a	50.4±0.95 ^a	45.53±2.21 ^a
4	GT	16±1.15 ^a	26.67±0.88 ^a	22±1.15 ^a
5	CUR+GT	30.07±3.37 ^a	47.6±1.34 ^a	37.17±2.55 ^a
6	CIP	12±1.15 ^a	24.67±1.45 ^a	20±1.15 ^a
7	G	21.83±5.51 ^a	47±1.1547 ^a	42±0.58 ^a
8	CUR+GT+CIP	26.67±6.67 ^a	46±2.08167 ^a	36.67±3.53 ^a

*Duncan's test represents differences between different groups at probability $p < 0.05$. Means with different superscripts (a, b, c, and d) within a column are profoundly different at $p < 0.05$. Values are given as the mean \pm SE .NC: non-treated non-challenged group; PC: Challenged with *E. coli* O78; CUR: Challenged with *E. coli* O78 and treated with curcumin in drinking water (200mg/L) from day one to of the trial; GT: challenged with *E. coli* O78 and treated with green tea in drinking water(400 mg/L) from day one of the trial;CUR+GT:Challenged with *E.coli* and treated with both curcumin and green tea;CIP:challenged with *E.coli* and treated with ciprofloxacin for 3 days post challenge;G:Garlic extract(500 mg/L) from day one to of the trial.

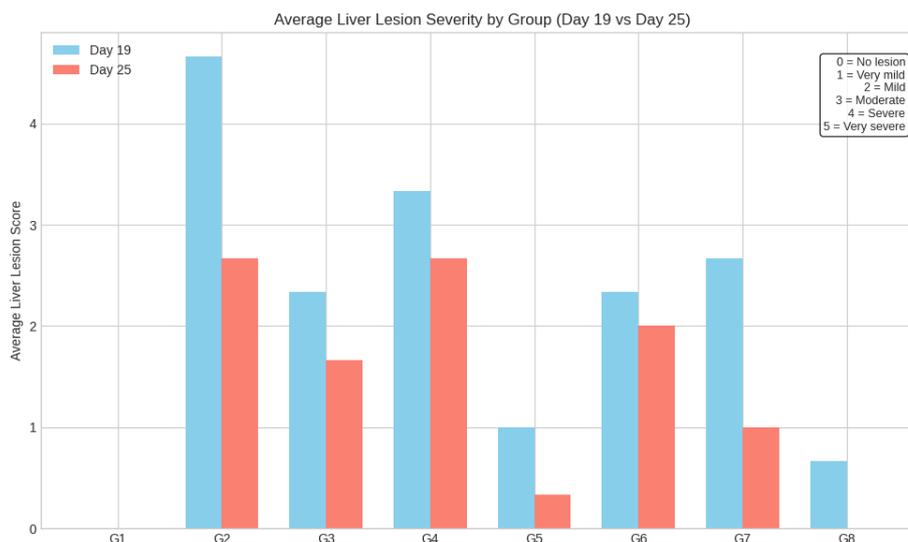


Fig. 1. evaluation of liver lesion scoring in on day 19 and day 25 according to [5]. Lesion Scoring Scale: 0 = No lesion; 1 = Very mild; 2 = Mild; 3 = Moderate; 4 = Severe; 5 = Very severe.G1 (NC): Negative Control; G2 (PC): Positive Control; G3 (CUR): Curcumin; G4 (GT): Green Tea; G5 (GT+CUR): combination of Green Tea and Curcumin (full doses); G6 (CIP): Ciprofloxacin; G7 (G): Garlic; G8 (GT+CUR+CIP): Triple regimen consisting of full doses of GT and CUR with a half-dose of CIP. Day 19 represents the peak infection period (5 days post-infection), while Day 25 shows the resolution phase (11 days post-infection).

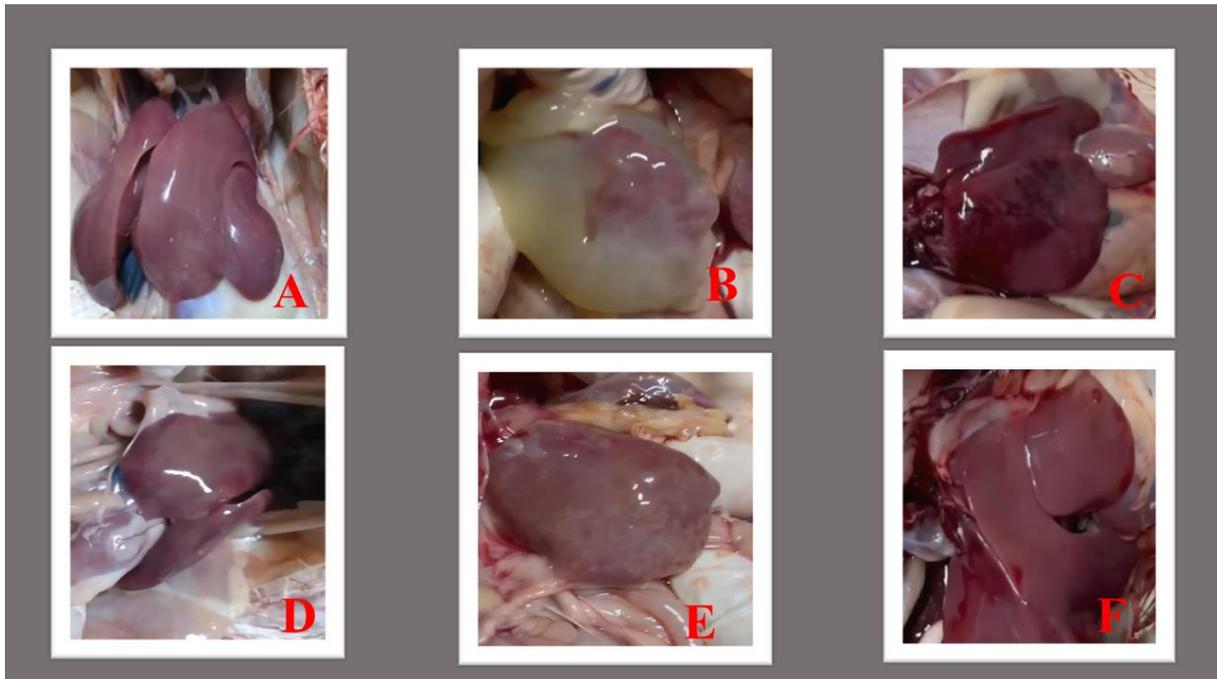


Fig. 2. Post-mortem examination of the liver (A) reveals no discernible lesions, indicating a normal liver. (B) A very severe liver lesion exhibiting a significant quantity of fibrinous exudate and perihepatitis. (C) A severe liver lesion demonstrating pronounced congestion and subcapsular bleeding. (E) A moderate lesion presenting necrotic foci within the liver. (D&F) exhibiting mild and extremely mild lesions with slight congestion, approaching a normal organ morphology.

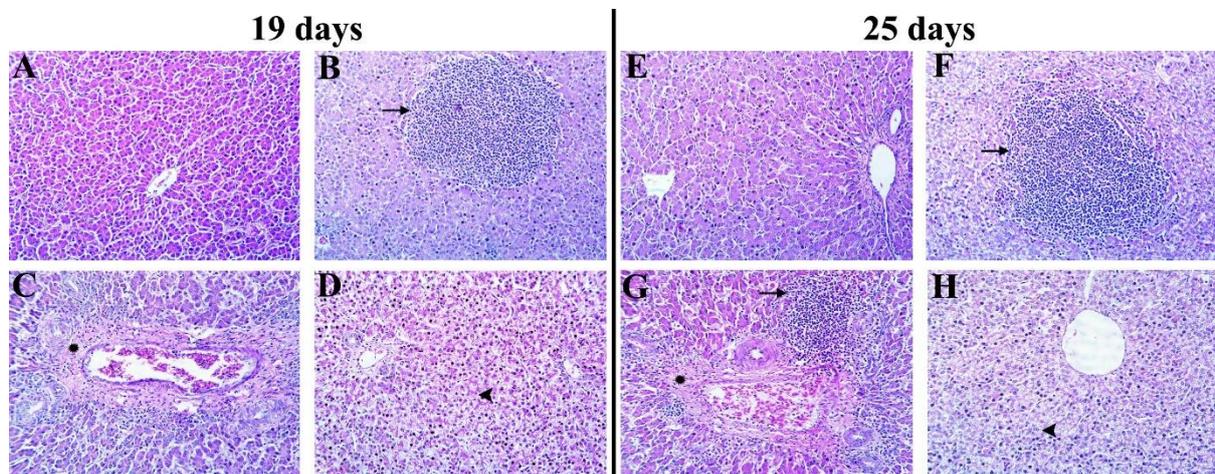


Fig. 3. Histological sections of liver tissue in poultry, H&E staining, x200 magnification. (A, E) Control group showing normal hepatic architecture; well-organized hepatic lobules, central veins, blood sinusoids and hepatocytes. The *E. coli* infected group showing extensive pathological alterations at both sacrifices including, focal lymphocytic cell aggregation (arrow) in (B and F), perivascular fibrosis (asterisk) accompanied with inflammatory cellular infiltration (arrow) (C and G), significant hepatocytes vacuolation (arrowhead) in (D and H).

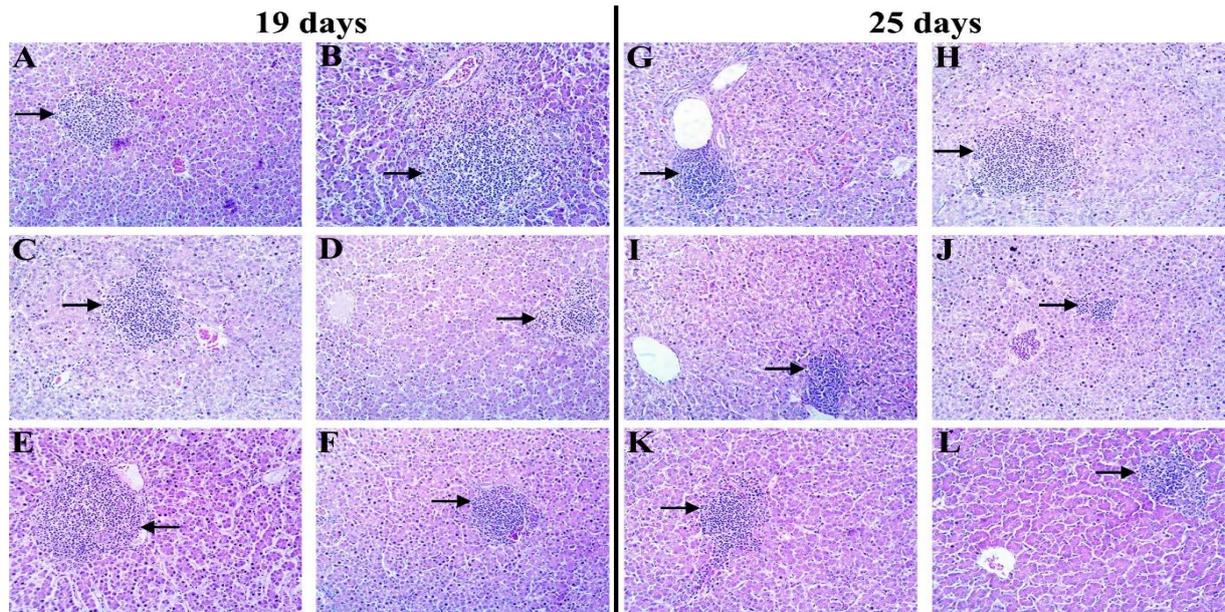


Fig. 4. Histological sections of liver tissue in poultry, H&E staining, x200 magnification. (A, G) curcumin group; (B, H) green tea group; (C, I) curcumin and green tea combination group; (D, J) curcumin, green tea, and ciprofloxacin group; (E, K) garlic treatment group; (F, L) ciprofloxacin group. All treatment groups exhibited improved liver pathology, especially lymphocytic cell aggregation (arrow) compared to the *E. coli* group with reduced hepatocellular degeneration and maintained liver architecture.

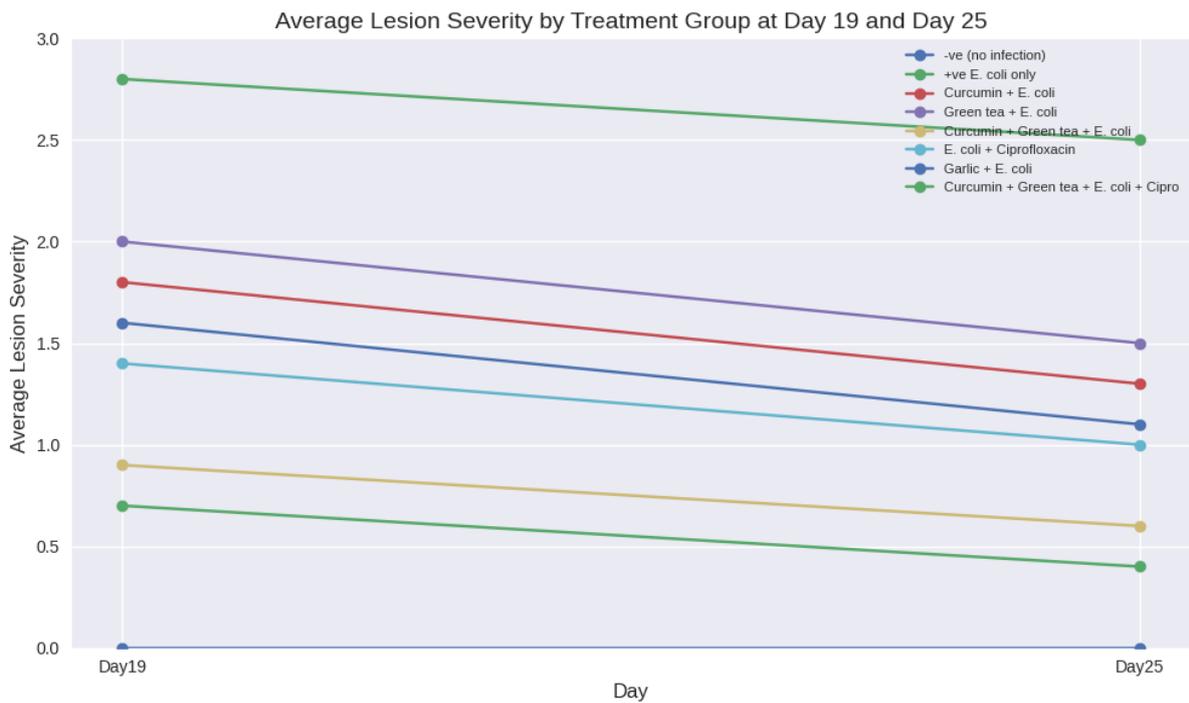


Fig 5. Histopathological evaluation of liver tissue was conducted at 19- and 25-days post-infection (dpi) This study employed a standardized ordinal scale of 0 to 3 for the scoring of histopathological changes, where 0 indicated the absence of lesions, 1 represented slight lesions, 2 denoted moderate lesions, and 3 signified severe lesions, in accordance with the methodology developed by [27].

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تقييم البدائل العشبية للمضادات الحيوية: تأثير الكركمين والشاي الأخضر ومستخلصات الثوم على النمو وصحة الكبد والإجهاد التأكسدي في الدجاج المصاب ببكتيريا الإشريكية القولونية

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الملخص

يشكل داء الإشريكية القولونية الطيري، الناجم عن الإشريكية القولونية الممرضة للطيور (APEC) من النمط المصلي O78، تهديداً كبيراً لإنتاج الدواجن نظراً لارتفاع معدلات الإصابة والنفوق والخسائر الاقتصادية. وبينما تبقى المضادات الحيوية، مثل سيبروفلوكساسين، العلاج الأساسي، فإن تزايد مقاومة المضادات الحيوية يستدعي تقييم علاجات بديلة. هدفت هذه الدراسة إلى مقارنة التأثيرات المضادة للبكتيريا، والمضادة للأكسدة، والتأثيرات النسيجية المرضية للكركمين والثوم والشاي الأخضر ومزيجها ضد عدوى تجريبية بالإشريكية القولونية O78 في دجاج التسمين، مع استخدام سيبروفلوكساسين كمييار مرجعي. تم توزيع 240 كتكوتاً عشوائياً على ثماني مجموعات، شملت مجموعات ضابطة سلبية وإيجابية، ومجموعات الكركمين، والشاي الأخضر، والثوم، والسيبروفلوكساسين، ومجموعات العلاج المركبة. تم تعريض الطيور (باستثناء مجموعة التحكم السلبية) لجرعة فموية من بكتيريا الإشريكية القولونية O78 في اليوم الرابع عشر. وتم تقييم العلامات السريرية، ومعدل الوفيات، ومعدل النمو، ودرجة الإصابة، والتغيرات النسيجية المرضية، ومؤشرات مضادات الأكسدة (SOD، CAT). أظهرت مجموعة التحكم الإيجابية إصابة شديدة ببكتيريا الإشريكية القولونية مع تلف كبدي ملحوظ، بينما أدت جميع العلاجات النباتية إلى تحسن سريري ونسجي مرضي واضح. قلل الكركمين والشاي الأخضر والثوم من شدة الإصابة بحلول اليوم التاسع عشر، وأظهرت مزيداً من الشفاء بحلول اليوم الخامس والعشرين، مع توفير الكركمين مع الشاي الأخضر حماية مبكرة ملحوظة. حقق مزيج السيبروفلوكساسين مع المستخلصات النباتية بنصف الجرعة أفضل تحسن في الإصابة. أظهرت نتائج مضادات الأكسدة نشاطاً أعلى لإنزيمي SOD و CAT في العلاجات القائمة على الكركمين، بينما سجلت مجموعة السيبروفلوكساسين ومجموعة التحكم المصابة أدنى القيم. من الناحية النسيجية، أظهرت جميع المجموعات المعالجة بنية كبدية محفوظة في الاستنتاجات، وقد خففت العلاجات النباتية من شدة الآفة، وقللت من الجرعة المطلوبة من المضادات الحيوية، وعززت حالة مضادات الأكسدة في الطيور المصابة ببكتيريا الإشريكية القولونية، لذا يمكنها تعزيز أو استكمال العلاج التقليدي بالمضادات الحيوية.

الكلمات الدالة: الإشريكية القولونية O78، دجاج التسمين، المستخلصات العشبية، مقاومة المضادات الحيوية، الإجهاد التأكسدي، الكركمين.