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Effects of Olive Leaf Extract on Growth Performance and Immunobiochemical Parameters in Turkey Poults

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ABSTRACT

Olive leaf extract (OLE) is known to have numerous bioactivities attributed to its high phenolic compound content. This study aimed to investigate the impact of OLE and Ceftriaxone on *Escherichia coli (E. coli)* in turkey poults. A total of 150 cloacal swabs were taken from turkey poults for isolation and identification of *E. coli*. Fifty-one-day-old turkey poults were divided into five equal groups. The first group served as the control, and the second group orally received 400 mg/kg body weight OLE daily for 35 days. The third, fourth, and fifth groups were infected with a culture suspension of *E. coli* O78 (0.3 ml, 3×10^7 organism/ml) via the nasal route. The third group was infected untreated. The fourth group was treated with 50 mg/Kg body weight of Ceftriaxone for 5 consecutive days. The fifth group received 400 mg/kg body weight of OLE from day to day 35 of age. Bacteriological examination revealed positive swabs in 18.18%, 46.67%, and 53.33% of healthy, diseased, and recently deceased poults, respectively. Serological identification of *E. coli* isolates included O157 (2), O78 (2), and O11 (1). Poults of the third group showed typical clinical signs, gross pathological changes such as congestion in various organs, and a 30% mortality rate. Additionally, significant reductions in body weight, weight gain, catalase (CAT), and superoxide dismutase (SOD) were observed, alongside anemia, hypoproteinemia, and hypoalbuminemia. Conversely, significant increases were noted in the phagocytic index, killing percentage, total globulin, immunoglobulins, and the albumin/globulin ratio. Furthermore, significant increases were observed in FCR, leukocytic counts, lysosome, tumor necrosis factor α (TNF-α), interleukin-10, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, creatinine, and malondialdehyde (MDA) levels. Poults in the fourth and fifth groups showed fewer clinical signs, lower lesion scores, and reduced mortality rates. Additionally, there was a decrease in *E. coli* re-isolation, modulation of altered parameters, and improvement in pathological lesions compared to the infected, untreated poults. Both OLE and Ceftriaxone were found to modulate the haematological, biochemical, and immunological parameters, as well as mitigate performance changes and pathological lesions induced by *E. coli* infection in turkey poults.

Keywords: Blood parameter, Ceftriaxone, *E. coli*, Olive leaves extract, Performance, Turkey poult

INTRODUCTION

Turkey is an important poultry species in Egypt, ranking second only to chickens in global meat production. However, turkeys are susceptible to various diseases, including *Escherichia coli* (*E. coli*), which poses a substantial threat to the turkey industry and results in considerable economic losses (Guabiraba and Schouler, 2015). Although *E. coli* is a commensal bacterium in the alimentary tract of healthy birds, it is also recognized as one of the main bacterial pathogens affecting turkeys (Rosario

et al., 2004). *E. coli* is a rod-shaped, Gram-negative, non-spore-forming bacterium causing high mortality and economic losses in the poultry industry. *E. coli* secretes lipopolysaccharide molecules, which cause acute inflammatory reactions and induce hematobiochemical and pathological alterations (Mohamed et al., 2022). Lipopolysaccharides existing in the bacterial cell wall cause cell and tissue injury as well as multiple organ dysfunction (Van Amersfoort et al., 2003). Colibacillosis, instigated by *E. coli*, is one of the leading causes of morbidity and mortality in poultry worldwide, acting either as a primary or secondary pathogen (Lutful Kabir, 2010).

In veterinary medicine, cephalosporins are commonly used because of their broad-spectrum activity and safety profile. Among these, Ceftriaxone is widely used in clinical practice due to its excellent antibacterial efficacy (Ghandour et al., 2023). Ceftriaxone, a broad-spectrum antibiotic belonging to the cephalosporin class, exhibits strong activity against both Gram-positive and Gram-negative bacteria by inhibiting bacterial cell wall synthesis (Prescott, 2013). However, the extensive use of antibiotics in the treatment of bacterial infections in humans and animals in recent decades has increased the percentage of antibiotic-resistant bacterial species in various environments. This trend has posed significant challenges for the selective treatment of bacterial infections (Fazeli-Nasab et al., 2021). Consequently, there is a growing need to explore alternative antibacterial agents derived from traditional medicine.

Olive leaves, which are rich in oleuropein phenols and flavonoids, are utilized in Mediterranean traditional medicine (Sedef and Sibel, 2009). Olive leaf extract (OLE) possesses antioxidant, anti-inflammatory, and antimicrobial properties (Anusha and Mohamed, 2013). OLE contains several potentially bioactive compounds classified as secoiridoids (e.g., oleuropein), flavonoids including flavones (e.g., apigenin and luteolin), flavonols (e.g., rutin and quercetin, and catechin), and simple phenols (e.g., tyrosol, hydroxytyrosol, vanillin, vanillic acid, gallic acid, caffeic acid, and verbascoside) (Sedef and Sibel, 2009). Phenolic compounds extracted from olive leaves might be beneficial to broilers through their antimicrobial activity against intestinal pathogenic bacteria (Sarica and Ürkmez, 2016). Methanol solvent olive extract is highly effective against *E. coli*. Due to the increasing resistance of bacteria to chemical antibiotics, antibacterial compounds from olives and other plants are used in the treatment of bacterial infections (Fazeli-Nasab et al., 2021). Therefore, this study aimed to investigate the comparative effects of olive leaf extract and Ceftriaxone on growth performance, immune response, and biochemical markers in turkey poults infected with *E. coli.*

MATERIAL AND METHODS

Ethical approval

This animal protocol was approved by the Agriculture Research Center Institutional Animal Care and Use Committee (ARC-IACUC) under protocol number ARC-AHRI-79-23, Egypt.

Bacteriological examination and serological identification of isolated *E. coli*

A total of 150 cloacal swabs were aseptically taken from 150 poults (110 healthy, 20 diseased, and 20 freshly dead). The swabs were inoculated into the nutrient broth and incubated at 37°C for 12 hours, followed by subculturing on MacConkey agar and nutrient agar plates for 24 hours at 37°C. Bacterial colonies were identified using standard microbiological methods (Mahon et al., 2018). Isolated *E. coli* were serotyped using slide agglutination test against polyvalent and mono-valent standard serum obtained from Denka Seiken Co., Ltd., Tokyo (Boop et al., 1999).

Antibiotic sensitivity test for isolated *Escherichia coli* (*In vitro*)

Using screening and confirmatory assays recommended by CLSI (CLSI, 2020), isolated *E. coli* O78 was examined for OLE by disc diffusion in comparison to several antibiotics.

Drugs

Ceftriaxone (Ceftriaxone^{R}), supplied by Pharco Company, Egypt, was used for intramuscular or intravenous injection in strengths equivalent to 250 mg, 500 mg, and 1000 mg of Ceftriaxone sodium.

Preparation of watery olive leaf extract

Fresh olive leaves were collected, washed with water, dried, and ground into a fine powder. A total of 7 g of the powder was mixed with 200 ml of boiling distilled water, left to steep, and then filtered. The filtrate was dried in an incubator at 35-40°C (Sylvia et al., 2003).

Experimental diet

The diets used throughout the 35-day experimental period and the physical composition of feedstuff are detailed in Tables 1 and 2.

Table 1. Formulation of experimental diet used for turkey poults during the experiment and the calculated chemical analysis

Ingredient		Calculated chemical analysis	
Ground yellow corn	50.7 kg	Crude protein (%)	26.1103
Soybean meal	32.4 kg	Ether extract (%)	3.687
Fish meal	5.9 kg	Crud fiber (%)	3.602
Corn gluten 60%	6.3 kg	Ca (%)	1.307
Soybean oil	1.1 kg	available Phosphorus (%)	0.549
Lysine Hcl78%	0.1 kg	Metabolic energy (Kcal /Kg)	2916.15
DL- methionine 98%	0.2 kg		
Calcium dibasic phosphate	1.4 kg		
Calcium carbonate	1.7 kg		
Premix	0.1kg		
Toxinil	0.1 kg		
Total	100		

Crude protein percentage and ether extract percentage were chemically analyzed, source: AOAC (1990), and calculated according to the feed composition, source: NRC (1994).

Table 2. Physical composition of feedstuff used in the formulation of diets during the experimental period.

Nutrient (% as fed basis) / Ingredient	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ca (%)	Available Ph (%)	Metabolic energy (Kcal /Kg)
Ground Yellow corn	7.9	3.5	2.2	0.05	0.1	3350
Soybean meal	44	1.2	7.3	0.35	0.27	2230
Fish meal	65	5	1	0.3.73	2.43	2580
Corn gluten 60%	60	2.4	1.3	0..07	0.14	3720
Soybean oil	0.0	00	00	0.0	0.0	8800
Calcium dibasic phosphate	00	00	00	21.3	18.5	00
Calcium carbonate	00	00	00	38	00	00
Lysine Hcl78%	118	0.0	0.0	0.0	0.0	4600
DL- methionine	58	0.0	0.0	0.0	0.0	3600

Ca: Calcium Ph: Phosphorus Kcal/kg: Kilo calories/ kilogram

Turkey poults and experimental design

Fifty one-day-old turkey poults, confirmed to be free from bacterial infection, were divided into five equal groups: GP1 included healthy poults (negative control); GP2 had healthy poults receiving 400 mg/kg body weight of aqueous olive leaf extract orally for 35 successive days (Atef and Fawziah, 2019; Erener et al., 2020). At day 30 of age, poult sin groups GP3, GP4, and GP5 were inoculated with 0.3 ml of a culture suspension of isolated *E. coli* O78 (3×10^7 organism/ml) via the nasal route (Nakamura et al., 1992). Poults in GP3 were infected with *E. coli* (positive control), and poults in GP4 were infected with *E. coli* and treated with 50 mg/Kg body weight of Ceftriaxone for 5 successive days (Pardeep et al., 2011). Moreover, poults in GP 5 received aqueous OLE for 35 days and were infected with *E. coli* at 30 days of age. Five poults from each group were individually weighed on the first day and day 36 to determine the weight gain and feed conversion ratio (FCR). Mortality rates, clinical signs, and post-mortem lesions were recorded. At 36 days of age, five poults from each group were sacrificed. Samples from the ileum, caecum contents, and fecal matter were collected in sterile bags, incubated on nutrient broth at 37°C for 24 hours, and then subcultured on nutrient agar (Woldehiwet et al., 1990). Isolated bacteria were identified using standard methods (Adams and Moss, 1999) and counted according to AOAC (1990).

Three blood samples were collected from each group at 36 days of age. The first blood sample was collected in Ethylenediaminetetraacetic acid anticoagulant (EDTA) tubes for hematological examination (Feldman et al., 2000). The second blood sample was taken in heparinized tubes for the estimation of phagocytic percentage and killing percentage (Wilkinson, 1976; Lee and Bacon, 1982). The third blood sample was collected without anticoagulant to obtain serum for the measurement of total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, and creatinine using a 7150 Automatic Blood Chemistry Analyzer (Ciba–Corning Diagnostic Crop). Additionally, serum levels of superoxide dismutase (SOD) (Nishikimi et al., 1972), catalase (CAT)

(Sinha, 1972), malondialdehyde (MDA) (Nielsen et al., 1997), immunoglobulin (IgA, IgG, and IgM) (Erhard et al., 1992), lysozyme activity (Schltz, 1987), interleukin-10 (IL-10), and tumor necrosis factor α (TNF-α) were measured using ELISA kits (WKEA MED Supplies) according to the manufacturer's instructions, using purified IL-2 and TNF-α antibodies, respectively.

Histopathological examination

Liver, kidney, and intestinal samples were taken from groups infected with *E. coli* (3, 4, and 5) at 36 days of age. Tissue samples were preserved and fixed in 10% neutral buffered formalin and then processed using the routine paraffin embedding technique employing alcohol and dehydrated in graded ethanol (70-100%), xylol, and melted paraffin wax at 60˚C, sectioned at the 4-5 micron thickness, and stained with H&E technique for routine examination (Suvarna et al., 2013).

Statistical analysis

Data was analyzed using one-way analysis of variance (ANOVA) with the SPSS program (version 16). Duncan's Multiple Range Test was used for post-hoc comparisons (Tamhane and Dunlop, 2000). Statistical significance was set at $p < 0.05$.

RESULTS

Of 110 healthy poults, 20 positive swabs (18.18%) were divided into 8 single isolates and 12 mixed isolates. Among 20 diseased poults, 6 positive swabs (30%) were detected, including 5 single isolates and 3 mixed isolates. Of 20 recently dead poults, 8 positive swabs (40%) were distributed as 3 single isolates and 5 mixed isolates (Figure 1 and Table 3). Serological identification of the isolated *E. coli* strains revealed 2 O157, 2 O78, and 1 O11 serotypes (Table 4). The antibiotic sensitivity test demonstrated that *E. coli* was more sensitive to Ceftriaxone compared to other antibiotics used in the study (Table 5).

Healthy poults receiving OLE (GP2) exhibited a significant increase ($p < 0.05$) in body weight gain compared to the normal control group (GP1; Figure 2). Hematobiochemical analysis revealed nonsignificant changes in ($p < 0.05$) in red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), heterophils, lymphocytes, eosinophils, basophils, monocytes, phagocytic percentage, phagocytic index, killing percentage, total globulin, white blood cells (WBCs), immunoglobulins (IgG, IGA, IgM), lysosome activity, interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), albumin/globulin (A/G) ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, and creatinine levels. Additionally, significant increases in serum total protein and albumin and improvement in FCR were observed in GP2 compared to the control group (Tables 6- 9; Figures 2 and 3).

Poults suffering from colibacillosis (GP3) showed clinical signs which included diarrhea, depression, dropping wings, listlessness, respiratory signs, frothy exudate in eyes, and loss of weight, with a mortality rate of 30%. Postmortem examination revealed congestion of the intestine, liver, and kidneys, along with hepatitis, degenerative changes hisopathologically, and a significant decrease ($p < 0.05$) in the body weight gain in poults infected with *E. coli* (GP3; Figure 2). Hematobiochemical findings revealed significant decreases ($p < 0.05$) in RBCs, Hb, PCV, serum total protein, albumin, total globulin, A/G ratio, CAT, SOD and significant increases ($p < 0.05$) in FCR, WBCs, heterophils, phagocytic percentage, phagocytic index, killing percentage, IgG, IgA, IgM, lysosome, TNF-α, IL-10, AST, ALT, ALP, uric acid, creatinine, and MDA. *E. coli* was reisolated from all infected poults in comparison to control poults (Tables 7, 8, 9, and 10; Figure 3).

Infected poults treated with OLE or Ceftriaxone (GP4 and GP5) showed mild clinical signs, lesion scores, reduction of mortality rate to 10 percent, reduction of *E. coli* re-isolation (Figure 1), a significant increase ($p < 0.05$) in body weight gain, RBCs, HB, PCV, phagocytic percentage, phagocytic index, killing percentage, IgG, IGA, IgM, CAT, SOD, total protein, albumin, total globulin, A/G ratio, as well as non-significant changes ($p < 0.05$) in WBCs, heterophil, lymphocyte, basophil, eosinophil, monocyte, coupled with improvement in FCR, AST, ALT, ALP, uric, creatinine, lysosome, TNF-α, IL-10, and MDA ($p < 0.05$) in comparison to the infected poults.

Histopathological findings

The liver of turkey poults infected with *E. coli* (GP3) at the end of the experiment exhibited focal necrotic areas (Figure 4A), hydropic degeneration, and dilated sinusoids (Figure 4B). The kidneys showed severe peritubular hemorrhage (Figure 4C) and the deposition of intra-tubular eosinophilic substance with some degenerative changes (Figure 4D). Intestinal samples showed focal destruction of the intestinal muscularis mucosa (Figure 4E). In contrast, the liver of turkey poults infected with *E. coli* and treated with Ceftriaxone (GP4) at the end of the

experiment showed mild focal lymphocytic cell infiltration in hepatic parenchyma (Figure 5A). Kidneys exhibited mild focal tubular cloudy swelling (Figure 5B), while the intestine showed a mild increase in goblet cells (Mucinous degeneration; Figure 5C). Poults that received OLE and were subsequently infected with *E. coli* (GP5) demonstrated mild focal hemorrhage in the liver (Figure 6A). Kidneys showed hydropic degeneration of some renal epithelial cells (Figure 6B), and the intestine displayed long, fused intestinal villi (Figure 6C).

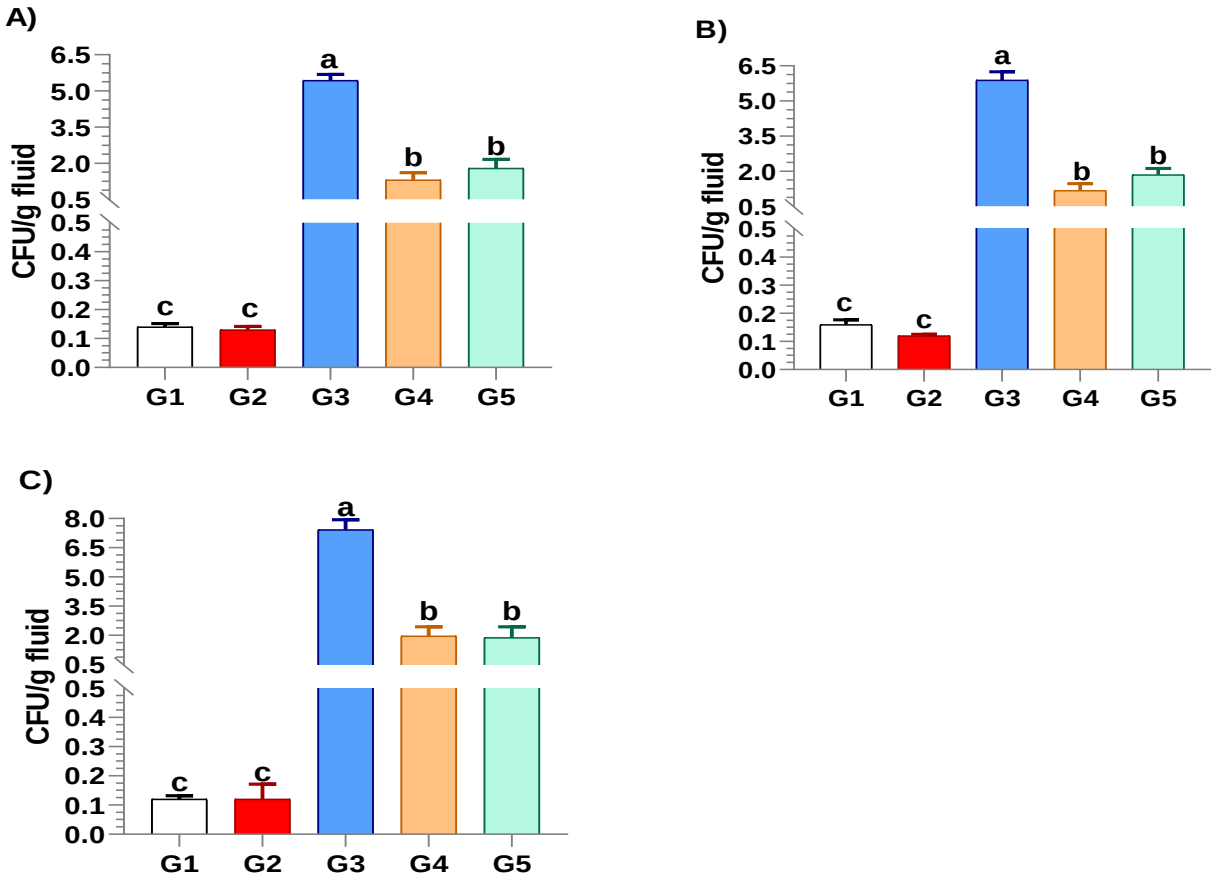

Figure 1. Effect of olive leaf extract and ceftriaxone on re-isolation and counts of *Escherichia coli* (CFU/g fluid) in Ileum (A), Caecum (B), and fecal matter (C) of pouts experimentally infected with *E. coli*. GP(1): Negative control GP(2): Treated with Olive leaf Extract GP(3): Infected with *E. coli* (positive control). GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 3. Prevalence of bacterial isolates from cloacae swabs obtained from turkey poults

	Healthy (total examined 110)				**Diseased (total examined 20)**				**Freshly dead (total examined 20)**			
+ veswab	**20 isolate (18.18%)**				**7 isolate (35%)**				**8 isolate (40%)**			
Type of isolate	Single 8(40%)		Mixed 12 (60)		Single 4 (28.8)		Mixed 3 (71.4)		Single 3 (37.5%)		Mixed 5 (62.5%)	
Isolate	*E. coli*	2	*E. coli* + *Staph Aureus*	5	*E. coli*	2	*Strept spp* + *E. coli*	1	*E. coli*	1	*Sal. spp*+ *E. coli*	1
	Sal. spp.	2	*E. coli*+ *Proteus*	3	*Strept.sp*	1	*Sal. sp*+ *E. coli*	1	*Staph aureus*	1	*E. coli*+ *Staph Aureus*	3
	Strep spp	4	*Sal. spp* +*E. coli*	4	*Sal spp*	1	*sal* .+ *E. coli*	1	*Sal.spp*	1	*Sal. spp*+*E.coli*	1

Sal. Spp.: Salmonella species Staph: Staphylococcus

Table 4. Serological identification of *Escherichia coli* isolated from turkey poults

Serotype	O157	O78	O11
Healthy (2)	1	1	-
Diseased (2)	1	1	-
Dead (1)	-	-	1

Table 5. Susceptibility of *Escherichia coli* to olive leaf extract and some antimicrobial agents

	Olive	Ceftriaxone	Erythromycin	Gentamycin	Florfenicol
Mark and potency	00	CRO (30mg)	Define under the table (30mg)	Gm (10mg)	FF (30mg)
Inhibition zone	17	24	21	15	21
sensitive	++	++++	+++	++	++++

CRO: Ceftriaxone; Gm: Gentamycin; FF: Florfenicol

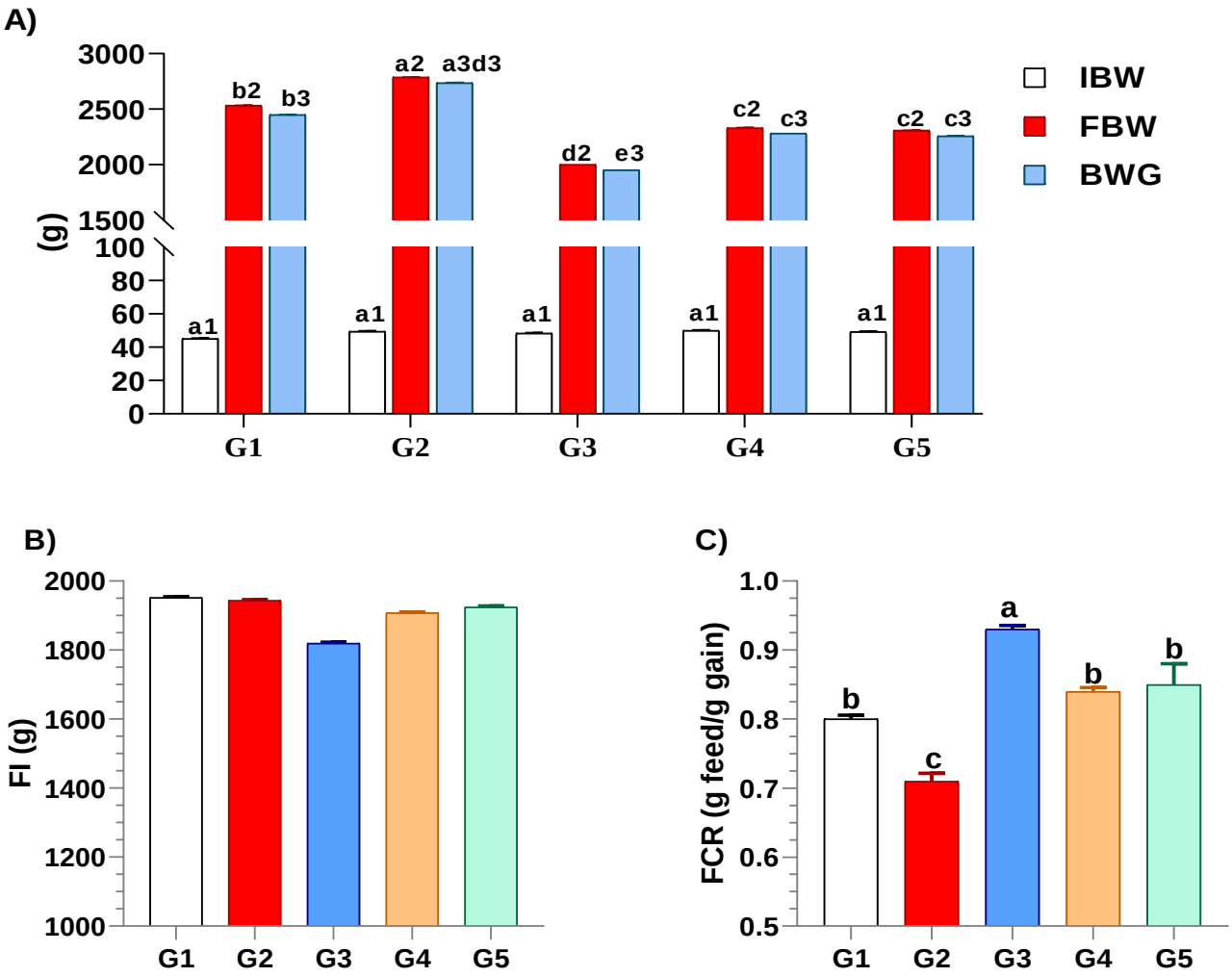

Figure 2. Effect of olive leaf extract and Ceftriaxone on body performance. **A:** Initial body weight, IBW; Final body weight, FBW; Body weight gain (BWG) and feed utilization. **B:** Feed intake, FI; Feed conversion ratio (FCR) in poults experimentally infected with *Escherichia coli*. GP(1): Negative control GP(2): Treated with olive leaf extract GP(3): Infected with *E. coli* (positive control) GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 6. Effect of olive leaves extract and ceftriaxone on hematology of poults experimentally infected with *Escherichia coli* at 36 days of age

Group	Erythrogram			Leukogram					
	RBCs (10^6/cu mm)	HB (g/dl)	PCV (%)	WBCs $X10^3$/µl	Differential leukocytic count (X103/µl)				
					Heterophil	Lymphocyte	Eosinophil	Basophil	Monocyte
Gp (1)	6.06 ± 0.31[a]	12.32 ± 0.17[a]	29.21± 0.28[a]	11.16 ± 0.38[b]	3.31 ± 0.13[b]	4.39 ± 0.32[a]	1.19 ± 0.13[a]	1.21 ± 0.15[a]	1.06 ± 0.12[a]
Gp (2)	6.01 ± 0.22[a]	12.67 ± 0.13[a]	29.01 ± 0.19[a]	11.44 ± 0.16[b]	3.20 ± 0.11[b]	4.48 ± 0.69[a]	1.38 ± 0.18[a]	1.27 ± 0.17[a]	1.11 ± 0.10[a]
Gp (3)	4.44 ± 0.09[b]	8.23 ± 0.27[b]	17.32 ± 0.12[b]	12.02 ± 0.33[a]	4.26 ± 0.29[a]	4.48 ± 0.88[a]	1.15 ± 0.07[a]	1.18 ± 0.06[a]	1.03 ± 0.09[a]
Gp (4)	5.79 ± 0.27[a]	11.89 ± 0.29[a]	28.88 ± 0.46[a]	11.82 ± 0.16[a]	4.28 ± 0.42[a]	4.49 ± 0.47[a]	1.16 ± 0.10[a]	1.19 ± 0.11[a]	1.04 ± 0.07[a]
Gp (5)	5.96 ± 0.19[a]	11.89 ± 0.07[a]	28.68 ± 0.51[a]	12.06 ± 0.05[a]	4.89 ± 0.51[a]	3.98 ± 0.59[a]	1.08 ± 0.11[a]	1.13 ± 0.10[a]	1.01 ± 0.09[a]

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. RBCs: Red blood cells, HB: Hemoglobin, PCV: Packed Cell Volume, WBCs: White blood cells. GP(1): Negative Control GP(2): Treated with olive leaf extract GP(3): Infected with *E coli*(positive control). GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE

Table 7. Effect of olive leaf extract and ceftriaxone on immunoglobulin of poults experimentally infected with *Escherichia coli*

Parameter	Immuonoglobuline (gm/100ml)		
Groups	**IgG**	**IgM**	**IgA**
Gp(1)	3.97 ± 0.89[c]	5.70 ± 0.57[c]	8.21 ± 0.91[b]
Gp(2)	4.12 ± 0.95[c]	6.66 ± 0.89[c]	8.77 ± 0.96[b]
Gp(3)	6.98 ± 0.89[b]	7.74 ± 0.23[b]	9.89 ± 0.78[b]
Gp(4)	6.91 ± 0.57[b]	6.87 ± 0.53[c]	9.81 ± 0.71[b]
Gp(5)	8.22 ± 0.78[a]	9.21 ± 0.62[a]	11.41 ± 0.87[a]

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. IgA: Immunoglobulin A IgM: Immunoglobulin M IgG: Immunoglobulin G. GP(1): Negative control GP(2): Treated with olive leaf extract GP(3): Infected with *E coli* (positive control) GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 8. Effect of olive leave extract and ceftriaxone on CAT, SOD, MDA, IL-10, and TNF-α, in poults experimentally infected with *Escherichia coli*

Parameter	Antioxidant enzymes		MDA	IL-10	TNF-α
Group	**CAT(U/mL)**	**SOD(U/mL)**	**(ug/ml)**	**pg/ml**	**pg/mL**
Gp(1)	9.37 ± 0.89[b]	25.55 ± 1.58[b]	9.58 ± 0.82[b]	0.89 ± 0.21[b]	0.99 ± 0.18[b]
Gp(2)	15.09 ± 0.63[a]	32.82 ± 1.21[a]	8.06 ± 0.94[c]	0.80 ± 0.18[b]	0.94 ± 0.15[b]
Gp(3)	7.68 ± 0.45[c]	23.65 ± 1.37[c]	10.94 ± 0.73[a]	0.94 ± 0.15[a]	2.84 ± 0.21[a]
Gp(4)	9.48 ± 0.66[b]	25.12 ± 1.83[b]	9.12 ± 0.98[c]	0.89 ± 0.19[a]	1.03 ± 0.16[b]
Gp(5)	14.21 ± 0.75[a]	30.17 ± 1.53[a]	9.73 ± 0.78[b]	0.91 ± 0.13[a]	1.02 ± 0.53[b]

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. CAT: Catalase SOD: Soper Oxide Dismutase MDA: Malonaldhyde IL-10: Interleukin 10 TNF-α: Tumer Necrosis Factor-α. GP(1): Negative Control GP(2): Treated with olive leaf Extract GP(3): Infected with *E coli* (positive control) GP(4): Infected with *E coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 9. Effect of olive leaf extract and ceftriaxone on liver enzymes and kidney function of poults experimentally infected with *Escherichia coli*

Parameters \ Groups	GP 1	GP 2	GP 3	GP 4	GP 5
Total protein	4.72 ± 0.41^{b}	5.93 ± 0.33^{a}	2.86 ± 0.30^{c}	4.01 ± 0.35^{b}	3.98 ± 0.34^{b}
Albumin	2.08 ± 0.21^{c}	3.00 ± 0.19^{a}	1.59 ± 0.17^{d}	2.10 ± 0.21^{b}	2.06 ± 0.19^{b}
Total globulin	2.64 ± 0.15^{a}	2.93 ± 0.19^{a}	1.37 ± 0.17^{c}	1.91 ± 0.21^{b}	1.88 ± 0.19^{b}
AG ratio	1.35 ± 0.19^{a}	1.33 ± 0.11^{a}	1.07 ± 0.16^{b}	1.36 ± 0.12^{a}	1.36 ± 0.08^{a}
AST	78.48 ± 0.98^{c}	76.39 ± 0.78^{c}	81.42 ± 0.82^{a}	79.75 ± 0.69^{b}	79.83 ± 0.91^{b}
ALT	37.32 ± 0.89^{c}	36.83 ± 0.54^{c}	40.61 ± 0.55^{a}	38.32 ± 0.71^{b}	38.41 ± 0.63^{b}
ALP	89.14 ± 0.79^{b}	87.87 ± 0.89^{b}	92.61 ± 0.77^{a}	90.33 ± 0.78^{b}	90.40 ± 0.55^{b}
Uric acid	5.41 ± 0.43^{b}	5.02 ± 0.43^{b}	8.12 ± 0.55^{a}	6.60 ± 0.75^{b}	7.66 ± 0.49^{ab}
Creatinine	1.15 ± 0.16^{b}	1.10 ± 0.13^{b}	1.89 ± 0.31^{a}	1.42 ± 0.27^{a}	1.50 ± 0.46^{a}

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. AG ratio: Albumin/Globulin Ratio. AST: Aspartate amino transferase ALT: Alanin amino transferase. ALP: Alkaline phosphatase. GP 1: Negative control, GP 2: Treated with olive leaf extract, GP 3: Infected with *E. coli* (positive control), GP 4: Infected with *E. coli* and treated with Ceftriaxone, GP 5: Infected with *E. coli* and treated with OLE.

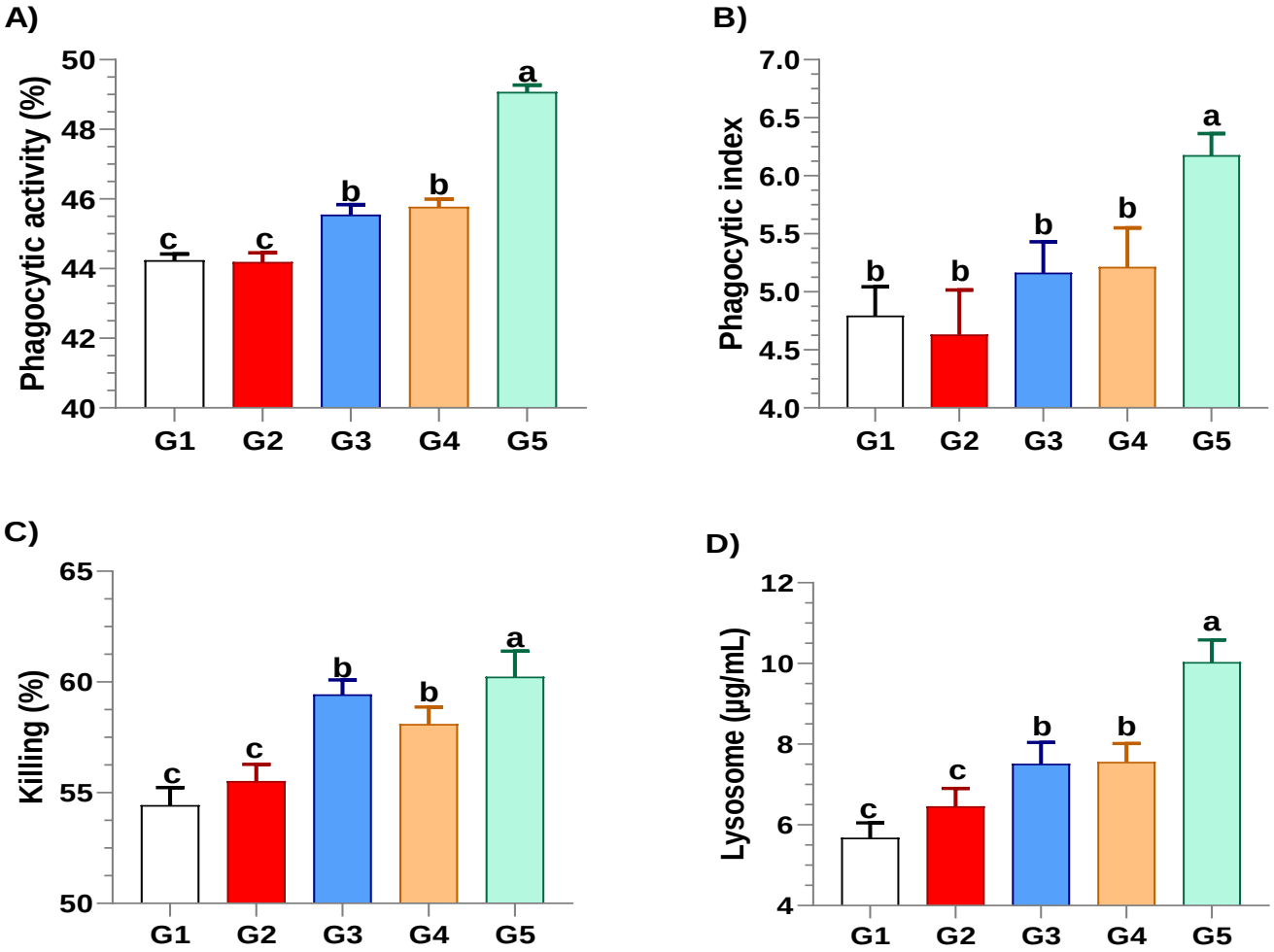

Figure 3. Effect of olive leaf extract and Ceftriaxone on phagocytic activity (A), phagocytic index (B), killing rate (C), and lysosome (D) in poults experimentally infected with *Escherichia coli*. GP 1: Negative control GP 2: Treated with olive leaf extract GP 3: Infected with *E. coli* (positive control) GP 4: Infected with *E. coli* and treated with Ceftriaxone GP 5: Infected with *E. coli* and treated with OLE.

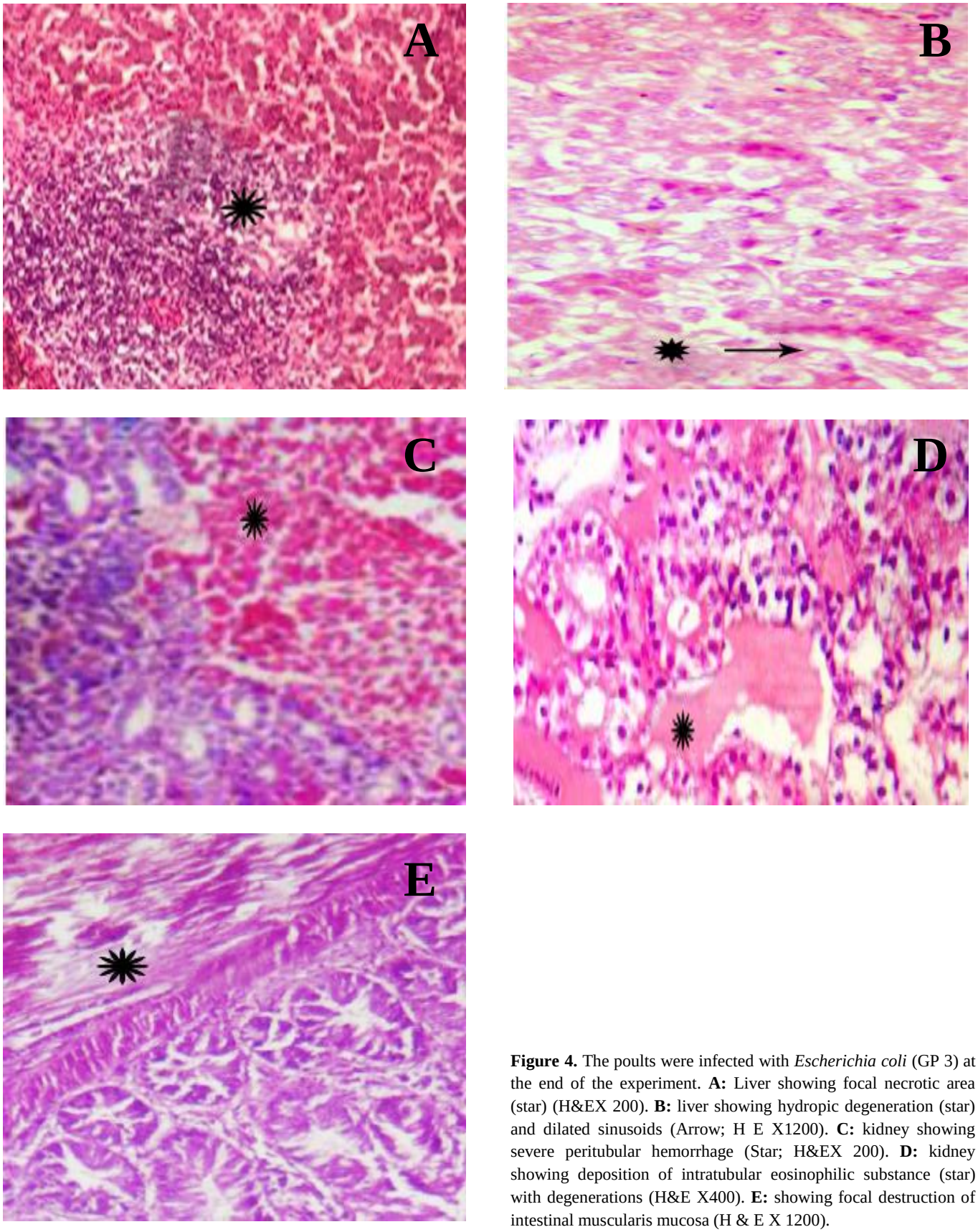

Figure 4. The poults were infected with *Escherichia coli* (GP 3) at the end of the experiment. **A:** Liver showing focal necrotic area (star) (H&EX 200). **B:** liver showing hydropic degeneration (star) and dilated sinusoids (Arrow; H E X1200). **C:** kidney showing severe peritubular hemorrhage (Star; H&EX 200). **D:** kidney showing deposition of intratubular eosinophilic substance (star) with degenerations (H&E X400). **E:** showing focal destruction of intestinal muscularis mucosa (H & E X 1200).

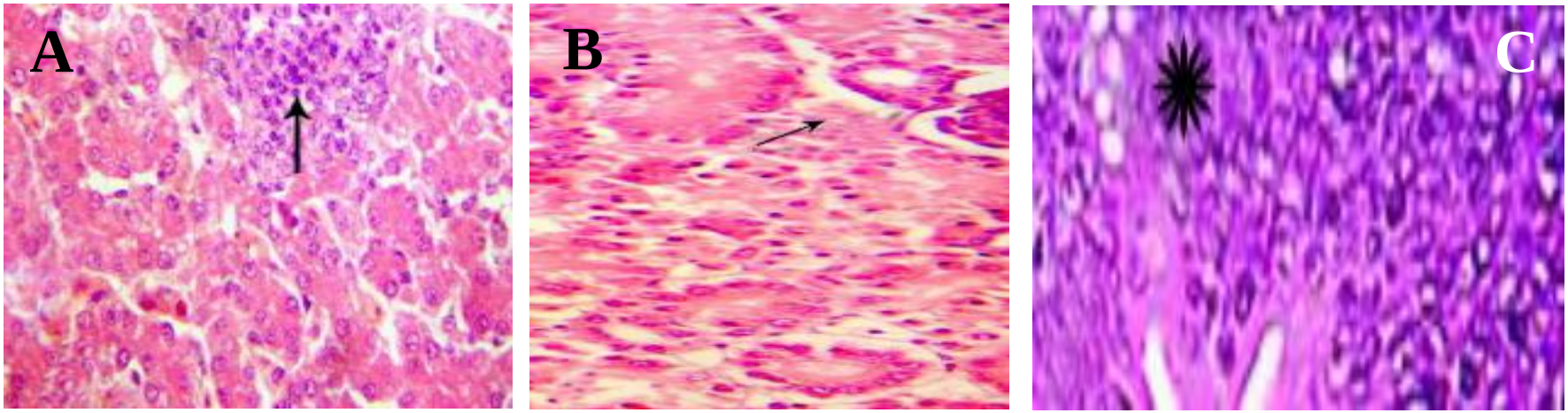

Figure 5. The poults infected with *Escherichia coli* and treated with ceftriaxone (GP 4) at the end of the experiment. **A:** liver showing focal mild lymphocytic cells infiltrated the hepatic parenchyma (Arrow; H&E, X1200). **B:** kidney showing mild focal tubular cloudy swelling (Arrow; H&E, X1200). **C:** The intestine shows a mild increase in goblet cells (Star; Mucinous degeneration ; H&E, X1200).

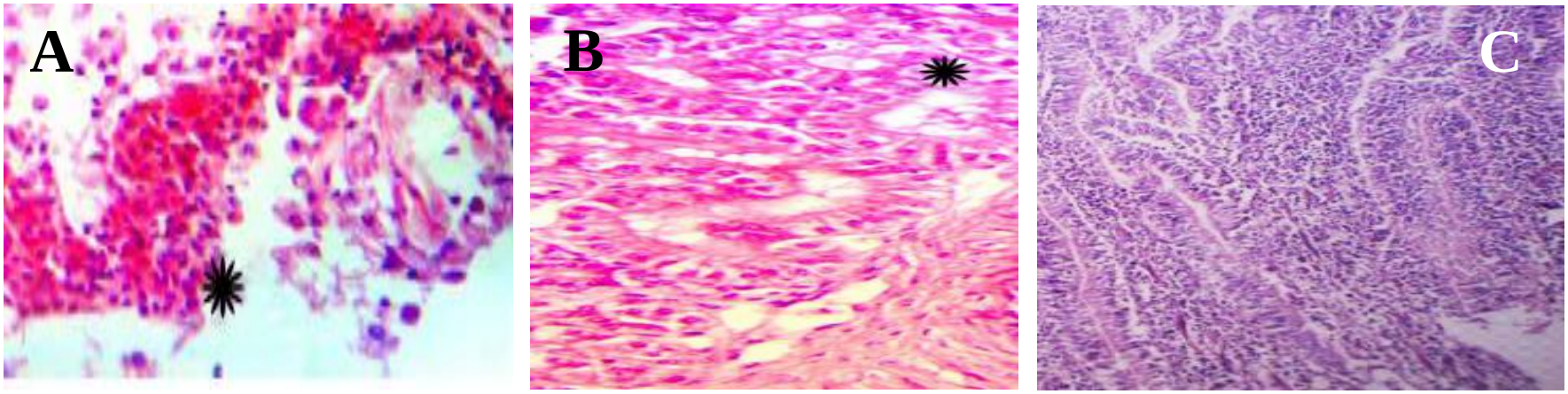

Figure 6. The poults receiving olive leaf extract and subsequently infected with *Escherichia coli* GP (5) at the end of the experiment. **A:** Liver showing mild focal hemorrhage (star) (H& E X1200). **B:** kidney showing hydropic degeneration of some renal epithelium (star), (H&E X300). **C:** Intestine showing long fused intestinal villi (H&E X300).

DISCUSSION

Avian colibacillosis, caused by *E. coli* (either as a primary or secondary infection), is one of the main causes of morbidity and mortality in poultry (Barnes et al., 2008). Moreover, there is a higher tendency to utilize medicinal herbs to alleviate diseases due to their reduced risk of side effects (Fazeli-Nasab et al., 2021). Certain herbs and medicinal plants, such as olives, have demonstrated beneficial effects on various physiological systems owing to their antioxidant properties (Cotelle et al., 1996). For instance, the disc diffusion test revealed that OLE induced an inhibitory zone of approximately 17 mm against isolated *E. coli*, with Ceftriaxone being the most effective antibiotic among those tested. Similarly, Gökmen et al. (2014) reported that OLE induced an 18 mm inhibitory zone against *E. coli*. Furthermore, *E. coli* has been shown to exhibit high sensitivity to ceftriaxone (Ghandour et al., 2023).

In the present study, poults infected with *E. coli* showed clinical signs including ruffled feathers, dropping wings, inappetence, depression, conjunctivitis, sneezing with frothy exudates in their eyes, diarrhea, and a mortality rate of 30%. These symptoms may be attributed to the effect of *E. coli* endotoxins on various organs. These results are partially consistent with those of El-Tahawy et al. (2022) in broiler chickens and align with Reham et al. (2021), who reported diarrhea, loss of appetite, mouth breathing, sneezing, ruffled feathers, weight loss, and a 30% mortality rate in chickens infected with *E.coli.*

The results of this study revealed a decrease in body weight gain and an increase in FCR as a result of inappetence, intestinal damage, poor digestion, and diarrhea. This finding was supported by El-Tahawy et al. (2022), who observed that experimental *E. coli* infection in broilers induced weakness, loss of appetite, depression, cough, and watery diarrhea in addition to a recorded mortality rate of 30% as well as a reduction in growth performance. The obtained results align with those of Stordeur and Mainil (2002), who stated that colibacillosis in broiler chickens induced low performance and weight gain.

Based on the current study, the postmortem examination of poults revealed congestion of the internal organs and hepatitis, likely caused by *E. coli* endotoxins. This finding is similar to that noted by Reham et al. (2021) and Ghandour et al. (2023), who observed the same postmortem lesions in chickens with *E.coli* infection.

In the current study, treatment of *E. coli*-infected poults with either OLE or Ceftriaxone resulted in improved clinical signs, reduced mortality, enhanced weight gain, and a decrease in re-isolation of *E. coli.* This improvement is likely due to the antimicrobial properties of OLE and ceftriaxone. These observations are consistent with the findings of Markin et al. (2003), who reported that OLE exhibited antibacterial effects against *E. coli*, reduced clinical signs, improved weight gain, and reduced re-isolation of *E. coli*. Similarly, Ghandour et al. (2023) demonstrated that Ceftriaxone was effective against *E. coli*, reducing clinical signs, eliminating mortality, improving weight gain, and reducing *E. coli* re-isolation in broiler chickens.

The obtained data revealed that healthy poults receiving OLE showed insignificant changes in RBCS, Hb, PCV%, and WBCs, as well as an insignificant increase in heterophils, lymphocytes, eosinophils, basophils, monocytes, phagocytic index, and killing percentage at the end of the experiment compared to healthy control poults. Similarly, olive leaf powder was found to induce elevation in RBCS, Hb, PCV %, and WBCs in El-Damarawy et al.'s (2013) study. The obtained results align with those of Ahmed et al. (2017), who stated that olive oil increased RBCS, Hb, PCV%, WBCs, lymphocytes, and neutrophils in rats and chickens.

In this study, the poults infected with *E. coli* showed a reduction of RBCs, Hb, and PCV and an increase in WBCs, heterophils, phagocytic index, and killing percentage, which may be due to *E. coli* endotoxins. These results corroborate with those of Allam et al. (2016), who stated that broilers infected with *E. coli* showed a reduction in RBCS, Hb, PCV, lymphocytes, monocytes, eosinophils, and basophils, as well as a significant increase in WBCs and heterophils. This finding aligns with El-Tahawy et al. (2022), who stated that *E-coli* induced a reduction in RBCS, Hb, and PCV in broilers. Moreover, Mithin et al. (2022) mentioned that broilers infected with *E. coli* showed an increase in WBCs and heterophils.

Poults infected with *E-coli* and treated with either OLE or Ceftriaxone showed a significant increase in RBCs, HB, PCV, phagocytic index, and killing percentage, coupled with a significant reduction in WBCs and heterophils in comparison with infected untreated poults. This may be due to the antibacterial effects of OLE against *E. coli*, leading to the improvement of the examined hematological and immunological parameters. The same observation was recorded by El-Kholany et al. (2022), who stated that OLE was effective against *E. coli* and improved the hematological parameters. Phenolic compounds extracted from olive leaves were found effective due to their antimicrobial activity in broilers

(Sarica and Ürkmez, 2016). Ceftriaxone was powerful in the treatment of colibacilosis and improved RBCS, Hb, PCV, WBCs, and phagocytosis (Mithin et al., 2022). These findings partially agree with Ghandour et al. (2023), who reported that broilers infected with *E. coli* and treated with Ceftriaxone showed an elevation in RBCS, Hb, and PCV together with a reduction in WBCs when compared to infected untreated broilers.

In the present study, poults suffering from colibacillosis showed a significant decrease in CAT and SOD, besides a significant increase in IgG, IgA, IgM, lysosome, IL-10, TNF-α, and MDA levels compared to healthy control poults. According to Coleman (2001), this might result from severe inflammation and oxidative stress caused by Colibacilosis. *Escherichia coli* infection induced inflammation and oxidative stress, causing damage to the internal organs and leading to an increase in the lysosome, MDA, IL-10 a, and TNF-α as well as a decrease in CAT and SOD (Huda et al., 2020; Mohamed et al., 2022).

Poults suffering from colibacillosis and treated with OLE or ceftriaxone showed a significant increase in IgG, IgA, IgM, CAT, and SOD as well as a significant decrease in the lysosome, IL-10, TNF-α, and MDA levels compared to infected untreated poults. This may be due to the antioxidant properties of OLE and the antibacterial effects of both Ceftriaxone and OLE. In the same line, Markin et al. (2003) reported that OLE possesses antibacterial effects against *E. coli* and enhances antioxidant enzyme levels. Additionally, OLE has been shown to improve antioxidant enzymes, immunoglobulins (IgG, IgA, IgM), and MDA levels, demonstrating its efficacy against various bacterial infections (Lee and Lee, 2010). In previous studies, adding olive leaves to turkey diets led to increased serum concentrations of polyphenols such as oleuropein, hydroxytyrosol, tocopherol, carotene, sitosterol, and triglycerides, which are responsible for antioxidant activity and protection from blood lipid oxidation (Moudache et al., 2016; Lins et al., 2018). Furthermore, treatment of colibacillosis with cefepime has been shown to improve TNF-α and IL-10 (Coleman, 2001). These findings are consistent with Huda et al. (2020), who reported that broilers infected with *E. coli* and treated by cephalosporin (cephradine) showed improvements in CAT, SOD, and MDA levels.

In this study, poults infected with *E. coli* displayed a significant reduction in the total protein, albumin, total globulin, and A/G ratio, together with a significant elevation in AST, ALT, ALP, uric acid, and creatinine. These changes may be attributed to inappetence, diarrhea, inflammation of the intestine, and histopathological damage to liver and kidney tissues. The significant elevation in the hepatic enzymes in infected poults is likely due to degenerative changes and necrosis of the hepatic tissues, leading to increased hepatic permeability and the subsequent release of excessive liver enzymes into the serum. These findings are consistent with those of Manimaran et al. (2003), who reported that *E. coli* infection in broilers induced a significant reduction in the protein profile due to the malabsorption of amino acids from the inflamed intestine. Turkeys infected with *E. coli* showed an increase in serum liver enzymes, uric acid, and creatinine (Huff et al., 2008). In addition, infection with *E. coli* induced a reduction in protein profiles in broiler chickens and poults (Reham et al., 2021; Mohamed et al., 2022).

Poults infected with *E. coli* and treated by either OLE or Ceftriaxone showed a significant increase in total protein, albumin, and total globulin as well as a reduction in AST, ALT, ALP, uric acid, and creatinine in comparison with infected untreated poults. This improvement may be due to the antibacterial effect of both OLE and Ceftriaxone. The present study supports the results of Osman and Tantawy (2017) and Ghandour et al. (2023), who stated that olive leaf had hepatoprotective effects and improved the protein profile and liver enzymes. Olive leaf extract demonstrated antibacterial effects against *E. coli* due to the presence of phenolic compounds and the improvement of liver and kidney function (Takó et al., 2020).

Compared with the normal control group, the histopathology of the liver of *E. coli* infected turkey poults (GP3) showed the congestion of hepatic blood vessels and hepatic sinusoids, and the hydropic degeneration of hepatic cells, Their kidneys showed severe peritubular hemorrhage, pale eosinophilic substance, degenerative changes, and focal destruction of intestinal muscularis mucosa. These changes may be due to endotoxins present in *E. coli*. This suggestion was reinforced by Huff et al. (2008), who reported congestion, coagulative necrosis in the renal and hepatic parenchyma, degenerative changes in the hepatocytes and epithelium lining of renal tubules, and leukocytic infiltrations in *E. coli* infected broilers and turkeys. Reham et al. (2021) reported that the liver, kidneys, and intestines of broiler chickens suffering from colibacillosis revealed severe pathological abnormalities, inflammation, congestion, and degenerative changes, along with the sloughing of epithelium.

In this study, poults receiving OLE and then infected

with *E. coli* (GP5) showed mild focal hemorrhages in the liver and mild hydropic renal degeneration in the kidneys. They also demonstrated long fused intestinal villi in the intestine. This improvement may be due to the antimicrobial and antioxidant effects of OLE, as discussed by Ahmed et al. (2017). OLE enhances the mitochondrial membrane to prevent the disintegration of liver cells, eliminates liver blood vessels and sinusoids, and prevents the secretion of more liver enzymes, as reported by Vahidi-Eyrisofla et al. (2019). Such beneficial effects of OLE in improving intestinal absorption and nutritional digestibility are similar to those reported by Ahmed et al. (2017), who stated the reduction in the proliferation of pathogenic microorganisms and the production of toxins. Olive leaves possess anti-inflammatory and gastroprotective properties that improve intestinal epithelial cells (Mahmoud et al., 2021). The results of the current study are similar to those found by Papadopoulos et al. (2023), who reported that the gross lesions and histological structure of liver incorporation of OLE had a beneficial effect.

The livers of turkey poults in GP4 showed mild lymphocytic cells infiltrated in the hepatic parenchyma. Moreover, cloudy swelling and mucinous degeneration of the intestine were observed in their kidneys. Our results are supported by El-Tahawy et al. (2022) in their study on broiler chickens infected with *E. coli* and treated with another cephalosporine (cefquinome), showing improvement in the pathological lesions in the liver and kidneys. Similar improvements in the pathological lesions were reported by Ghandour et al. (2023), who stated that broiler chickens infected with *E. coli* and treated with Ceftriaxone demonstrated mild lesions in the liver, kidneys, and intestine.

CONCLUSION

The use of the aqueous extract of olive leaves (OLE) as a prophylactic measure and Ceftriaxone as a treatment for experimental *E. coli* infection not only improves body weight gain and FCR but also alleviates the deleterious effects induced on hematological and immunological parameters. Additionally, both OLE and Ceftriaxone modulate the pathological lesions induced by *E. coli* in turkey poults. Further research is recommended to evaluate the effects of OLE on different turkey breeds, the use of varying doses, its effects on other parameters, and its effects on meat quality.

DECLARATIONS

Authors' contributions

All authors contributed to the study conception and design, material preparation, data collection and analysis, and funding. The experimental diet and growth performance were performed by Ghada M. El Khedr. The hematological and biochemical investigations were performed by Doaa I.A. Mostafa, Marwa M. Sarhan, and Sara A. Abd El Wahab. The microbiological investigation was performed by Shaimaa A. Abd El-kader. The histopathological studies and histomorphometric measurements were performed by Heba A. Ewis, and Mohammed Kassem participated in the design of the study, writing, and revision of the manuscript, and in the approval of its final draft. The first draft of the manuscript was written by all authors who commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The data and materials are available upon reasonable request from the authors.

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Competing interests

The authors declare that there are no conflicts of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by all the authors.

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