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Effects of pomegranate peel extract-coated zinc nanoparticles on antioxidant status, biochemical parameters, and pathological findings in broiler chickens infected by avian pathogenic *E. coli*

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ABSTRACT

E. coli infection (colibacillosis) is among the most prevalent and significant poultry diseases due to its role in causing decreased weight gain, mortality, and reduced flock uniformity, though *E. coli*, as a normal flora, can be present in birds' intestinal tracts; some strains may cause respiratory and systemic diseases, referred to as avian pathogenic *E. coli* (APEC). The objectives of the study were to evaluate the growth performance and physiological parameters of *E. coli-infected* chickens treated with pomegranate extract-loaded zinc nanoparticles (PPE-ZnO-NPs). One hundred and fifty broiler chickens were allotted into five groups, consisting of 30 birds per group. In group G1, the control birds were negative; in group G2, the control-positive infected birds were positive for *E. coli* O87; G3, the infected birds were supplied with drinking water containing 40 mg/L of PPE-ZnO-NPs; G4, the infected-treated with 80 mg/L of PPE-ZnO-NPs given by drinking water; G5, the infected-treated with 120 mg/L of PPE-ZnO-NPs given for 35 days. The bird performance and mortalities were monitored, and blood and tissue samples were collected for biochemical, antioxidant, and pathological analysis. The mean of serum total cholesterol, triglycerides, creatinine, and urea was significantly reduced ($P<0.05$) when treated with PPE-ZnO-NPs. The concentration of malondialdehyde (MDA) was significantly decreased ($P<0.05$), while superoxide dismutase (SOD), catalase (CAT) activities, and glutathione (GSH) were significantly increased ($P<0.05$) by the addition of PPE ZnO-NPs compared to the positive control. It could be concluded that supplementation of PPE-ZnO-NPs to broiler improved birds' physiological and antioxidant status, which were infected with *E.coli*.

1. INTRODUCTION

Colibacillosis is a bacterial infection caused by *E. coli*, which can affect both humans and animals, particularly in the gastrointestinal and urinary tracts. The disease is most prevalent in young animals, such as poultry, pigs, and calves, but it also poses risks to humans, especially in cases of foodborne outbreaks (Abu El Hammed et al., 2022). However, certain strains known as avian pathogenic *Escherichia coli* (APEC) possess virulence factors that enable them to cause extraintestinal infections (Hu et al., 2022). Colibacillosis can manifest in various clinical forms, including septicemia, pericarditis, perihepatitis, airsacculitis, and cellulitis. Among these, systemic and respiratory infections are particularly devastating in broilers, leading to increased mortality, reduced growth performance, poor feed conversion, and carcass condemnation (Kunert et al., 2015). Infection typically occurs through the respiratory tract or via breaches in the gastrointestinal mucosa, often facilitated by environmental stressors such as poor ventilation, high ammonia levels, immunosuppression, or co-infections with viral or mycoplasmal agents (Shosha et al., 2024; Elmeligy et al., 2024). Once in the bloodstream, *E. coli* can colonize multiple organs, including the liver, heart, and lungs, resulting in widespread tissue damage and inflammatory responses. Due to the increasing resistance of *E. coli* strains to commonly used antibiotics (Fotouh et al., 2024a), there is growing interest in alternative control strategies, including the use of natural antimicrobial agents, nanoparticles, and immunomodulatory compounds (Mahmoud et al., 2025).

Nanoparticles have gained significant attention as an alternative or adjunct to traditional antibiotics in combating *Escherichia coli* infections, especially in the context of antibiotic resistance. They provide multiple mechanisms of action, such as reactive oxygen species (ROS) generation, membrane disruption, and enhanced drug delivery, making them versatile tools in antimicrobial therapy (Mahmoud et al., 2025). Various types of nanoparticles, including metallic nanoparticles (silver, gold, copper, zinc oxide), polymeric nanoparticles, and lipid-based nanoparticles, have demonstrated antibacterial acativity against *E. coli* (Abdel-Kareem et al., 2025). Metallic nanoparticles like silver and zinc oxide are particularly effective in generating ROS that damage bacterial cells, while polymeric and lipid-based nanoparticles improve drug delivery by targeting bacterial infection sites (Shoker et al., 2025).

Zinc oxide nanoparticles (ZnO NPs) have shown promise in the treatment of *Escherichia coli* infections due to their antibacterial properties. They are effective against various

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bacterial strains, including both antibiotic-sensitive and antibiotic-resistant *E. coli* (Zaki et al., 2025).

The mechanisms by which zinc oxide nanoparticles exert their antibacterial effects include the generation of reactive oxygen species (ROS), which lead to oxidative stress and damage to bacterial cell components such as proteins, lipids, and DNA. This oxidative damage ultimately results in bacterial cell death (Salah et al., 2025b). Additionally, ZnO NPs can disrupt the integrity of the bacterial cell membrane, increasing permeability and causing cell lysis. Studies have demonstrated that ZnO nanoparticles can also inhibit biofilm formation, a crucial factor in *E. coli* pathogenesis. By preventing biofilm development, ZnO NPs enhance the effectiveness of antibiotics, making them a valuable adjunct in combination therapy (Soufy et al., 2016).

Pomegranate extract, derived from the fruit Punica granatum, has gained attention for its antimicrobial properties, particularly against *Escherichia coli*. The extract is rich in polyphenolic compounds, such as punicalagins, ellagic acid, and flavonoids, which contribute to its antibacterial effects (Dahham et al., 2010). Research has demonstrated that pomegranate extract (PPE) has several mechanisms through which it exerts its antibacterial effects, particularly against *Escherichia coli*. PPE can disrupt the bacterial cell membrane. The components of the extract interact with the membrane, increasing its permeability, which leads to the leakage of essential cellular contents and ultimately results in cell lysis (Elbarbary et al., 2023). Additionally, PPE generates reactive oxygen species (ROS) within bacterial cells. This oxidative stress can damage crucial cellular components such as proteins, lipids, and DNA, resulting in cellular dysfunction and death (Basu 2014). Furthermore, PPE can inhibit the formation of biofilms. Biofilms are protective layers that bacteria form to shield themselves from environmental stressors and antibiotics. By preventing *E. coli* from adhering to surfaces and forming biofilms, PPE enhances the effectiveness of antibacterial treatments (Naghma 2018).

Due to its antibacterial properties, pomegranate extract holds potential as a natural treatment option for *E. coli* infections, especially in the context of rising antibiotic resistance. Studies suggest that using pomegranate extract as a complementary therapy could enhance the effectiveness of conventional antibiotics (Fotouh et al., 2020). Pomegranate extract, rich in polyphenolic compounds, can serve as a reducing and stabilizing agent for synthesizing zinc nanoparticles. First, the polyphenols in the extract reduce zinc ions to form zinc nanoparticles. This process not only aids in the formation of nanoparticles but also enhances their stability and dispersibility in solution (Ifeanyichukwu et al., 2020). Once zinc nanoparticles are formed, they exhibit significant antibacterial activity against *E. coli*. The combination of pomegranate peel extract with zinc nanoparticles creates a synergistic effect, enhancing the overall antibacterial properties. The extract contributes additional mechanisms of action, such as disrupting the bacterial cell membrane and generating reactive oxygen species (Shoker et al., 2024).

The present study evaluates the impact of pomegranate peel extract-coated zinc nanoparticles on broiler chickens infected with pathogenic *Escherichia coli* via assessment of antioxidant status, biochemical parameters, and pathological changes.

2. MATERIAL AND METHODS

2.1. Animal Approval Ethics

The experimental protocol of this experiment was approved ethically and approved by the Faculty of Veterinary Medicine, Benha University Animal Research Ethics Committee (approved no. BUFVTM 22-09-23).

2.2. Solvents and reagents:

Ethanol (analytical grade) – used as a solvent for PPE extraction, purchased from Sigma Company.

Deionized water – used throughout all experimental procedures.

Zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$] – purchased from Sigma Company and used as the zinc source.

Sodium hydroxide (NaOH) pellets – used as the precipitating agent in the synthesis of ZnO nanoparticles, purchased from Sigma Company.

All chemicals and reagents were of analytical grade and used without further purification.

2.3. Nano Zinc Oxide Preparations

The process of creating Nano Zinc Oxide involves preparing a solution of zinc acetate and sodium hydroxide in deionized water. The mixture is stirred for 6 hours, separated, washed, dried, and calcined at 400°C for 6 hours to obtain nano ZnO particles. This process ensures complete precipitation and purity (Liu et al., 2007).

2.4. Preparation and characterization of pomegranate peel extract

To extract pomegranate peel extract, wash and dry the peels thoroughly. Blend the powdered peels into a coarse powder, add solvent (ethanol) to the mixture, and heat it to 60°C for 2-4 hours. This process helps extract desired phytochemicals like polyphenols, flavonoids, and tannins. Filter the mixture and store the extract in an airtight container, preferably in a refrigerator or freezer (Nasiriboroumand et al., 2018).

2.5. Coating with Pomegranate Peel Extract

The process involves dispersing nano ZnO particles in pomegranate peel extract solution, sonicating for 60 minutes, centrifuging, washing with extraction solvent, and drying in a mild oven or vacuum oven at 60°C. Pomegranate extract and zinc nanoparticles were combined in a ratio of 9:1.

Pomegranate extract zinc nanoparticles coated with TEM features were utilized to identify the morphological features. The functional groups in the nano compound were estimated by Fourier transform infrared spectroscopy FT-IR; JASCO (FTIR-6200). Average particle size distribution was estimated using the dynamic light scattering technique DLS; zeta sizer (Malvern, ZS Nano, U.K.) measured the particle size.

2.6. Acute toxicity

Dissolve PPE-Zn-ONPs in deionized water and shake, and administer by gavage to various groups of five chickens each at 0, 50, 100, 250, and 500 mg/kg body weight (Kim et al., 2014) Observe the chickens continuously for subsequently for 24 h to look for changes in behavior, toxicity, and/or death signs, and death latency. The chickens were provided with food and water for 14 days, and their daily consumption of food, intake of water, body weight, death, and alteration in appearance were all recorded. The median lethal dose (LD50), which was able to kill half the population of experimental chickens, was estimated by following the protocol of Zhang et al. (2009).

2.7. Bird Management

The birds were given a well-balanced ration according to NRC (1994) shown in Table 1. The diet was given as starter until the 14th day of age, after which the chickens were given grower diet that was given until the 28th day of age, after which the chickens were given finisher diet until the termination of the experiment (35th day of age). Birds were maintained in consistent conditions for 35 days.

Table (1) Composition of starter, grower, and finisher diets /170 kg. (NRC, 1994).

Ingredient	Starter	Grower	Finisher
Corn	52.4	53.8	56.2
Corn gluten	60	60	60
Limestone powder	0.153	0.152	0.155
Sodium chloride	0.271	0.222	0.206
Concentrate	10	10	10
Di-calcium phosphate	0.127	0.16	0.19
Vegetable oil	4	5.28	5.77
Soybeans	32.6	29.99	27.22
Lysin Hcl	0.191	0.157	0.083
DL-Methionine	0.246	0.234	0.18

2.8. Bacterial agent

Escherichia coli O78 was kindly gifted as a donation from the Faculty of Veterinary Medicine, Benha University, Egypt, Central Laboratory. The colonies of *E. coli* strain were grown on nutrient broth for 24 hours at 37 °C and viable number adjusted to 4 × 10 CFU/ml colony forming units CFU viable organism/ml by phosphate buffered saline (PBS) according to Macfaddin (1980).

2.9. Experimental design and animals

One hundred and fifty chicks, one-day-old (Ross 308), procured from a local hatchery, were used. The birds were raised in pens on straw litter and reared in standard hygiene level in a temperature- and humidity-controlled building. Water was always available, and the birds were fed ad libitum complete feed mixtures appropriate for the rearing phase, depending on the broiler's nutrient requirement. Chickens were allocated randomly into five groups of 30 chicks/group. Group (1) was kept as a control group and was orally given normal saline by oral gavage for 7 days (control negative). Group (2) was challenged at 7th day age by crop gavages with 4 × 10 CFU/ml/bird of *E. coli* O78 serogroup in PBS for 2 consecutive days as described by Awaad et al. (2019) and left untreated; group (3) challenged orally at 7th day and treated with 40mg/ L PPE-Zn-ONPs daily according to Shakal et al. (2024); group (4) challenged orally at 7th day and treated with 80 mg/L PPE-Zn-ONPs daily; group (5) challenged orally at 7 days and treated with 120mg/L PPE-Zn-ONPs daily. During the first week of the experiment, the temperature was 34-35^{O}C and decreased stepwise by 2-3^{O}C each week.

The chickens were kept under the same management, hygienic, and environmental conditions throughout the whole 5-week study. Vaccination was carried out on day 7 of age with Hitchner+IB, and on day 18th with Lasota. The Gumboro vaccination was carried out at days 7 and 15 of age. The birds were examined daily for mortality, and the number of dead birds was recorded.

2.10. Sampling and Biochemical Analysis

On days 21 and 35, four birds from each group were picked at random, and sampling was carried out. Five ml of blood were drawn from the wing veins and added to nonheparinized tubes. The blood coagulum samples were centrifuged at 4000 rpm for 15 minutes, and clear serum was separated and placed in a −20° C freezer for the next biochemical analysis. Liver enzyme activity (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) was determined by the kinetic method by using a Human Diagnostics Worldwide, 196 Wiesbaden, Germany kit as mentioned by Breuer (1996). Serum triacylglycerol was determined according to Tietze (1995). Serum total cholesterol was determined according to the method reported by Schettler and Nussel (1975). The level of creatinine was estimated according to Husdan and Rapoport (1968), and Urea was estimated by using the Urease-Berthlot Method according to Zuo et al. (2014).

2.11. Tissue specimen

Liver tissues were minced into small pieces, weighed, and briefly cut for a short period. They were homogenized with a glass homogenizer in 9 volumes of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C, then the resulting supernatant was utilized to determine the following parameters: the concentration of MDA in the liver was determined as per the method adapted by Abo-Aziza et al. (2022), superoxide dismutase (SOD) activity, and GSH activity were determined as per the method adapted by Elbarbary et al. (2023). Catalase activity was determined by the protocol described by Aebi (1974).

2.12. Histopathological examination

The liver of 3 birds per group (2 birds/replicate) that were killed was selected for histological analysis to examine the frequency of histopathological lesions. The histopathological preparation procedure. Briefly, tissues were sliced to 3–4 mm thick, fixed in 10% neutral buffered formalin, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. The paraffin blocks were sectioned with the help of a microtome in 4–6 µm thickness and stained with Hematoxylin and Eosin stain to view general tissue architecture (Suvarna et al. 2019). Hematoxylin and Eosin-stained sections were viewed under the microscope.

2.13. Statistical analysis

The data was summarized based on its quantitative or qualitative nature. Qualitative data were analyzed as percentages, while the quantitative data were analyzed as mean ± S.E. Differences between groups were determined by using one-way ANOVA, which was subsequently confirmed through Tukey's multiple comparisons test. To identify significance, a p-value < 0.05 was used. Statistical calculations were carried out using GraphPad Prism version 8.02, developed by GraphPad Software Inc., San Diego, California, USA.

3. RESULTS

3.1. Characterization of PPE-Zn-ONPs

The sizes of the PPE-Zn-ONPs were determined by Transmission electron microscopy (TEM) (Fig. 1). It was observed that the size of the nanoparticles was of average size 28.23 nm and emerged as spherical clusters in shape, but they continue to exist since, during installation, the majority of the material changed into an amorphous form.

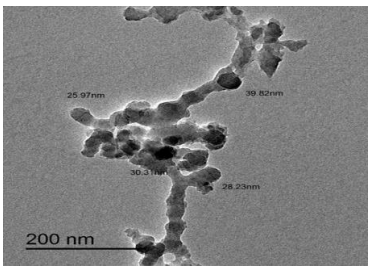

Fig. (1). Electron microscopy transmittance graphs of pom-ZnNPs

FTIR analysis was carried out to recognize different functional groups accountable for capping and stabilization of the PPE-Zn-ONPs (Fig. 2).

The different absorption peaks of the biosynthesized PPE-Zn-ONPs at 3309.59, 1905.75, 1638.05, 1085.21, 1045.51, 615.52, and 588.18 cm−1 are noticed in Fig. 2. The broad absorption band at 3309.59 cm−1 corresponding to O-H stretching of free hydroxyl groups can be attributed to the phenolic compounds of pomegranate peel extract. The peak at (1638.05) cm-1 for (C–N) stretching vibrations of aromatic amines, 1,045 cm-1 for primary alcohol C-O stretching of the functional molecules. Further, the absorption band at 1085.21 cm was due to C-O stretching of alcohols, a 1045 cm−1is representative of the -C-O stretch of alcohols, carboxylic acid esters, and ether functional groups. A peak of 588.18 cm−1 indicates the presence of the C-Br stretch of alkyl halides. These findings collectively imply the presence of functional groups in the synthesized ZnO-NPs, which is pertinent to their applications and functions

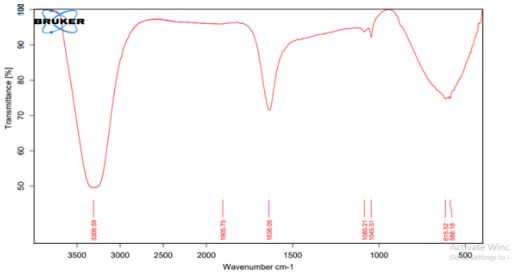

Fig. (2). The FT-IR spectrum of PPE-ZnNPs

3.2. Acute toxicity

No toxicity signs or mortalities were noted following oral treatment with the pom-ZnNPs at up to 500 mg/kg BW. In the current study, there were slight clinical signs of colibacillosis in all infected treatment groups. Like the chickens in group 2, the positive control group showed classical symptoms of colibacillosis three days after infection, such as weakness, depression, anorexia, dyspnea, coughing, sneezing, gasping, and nasal discharge. In Group 1, control negative, chickens that were active during the whole test period and did not show any sign of illness. All infected groups showed 100% morbidity but did not report any mortality.

3.3. Blood biochemistry parameters

Results indicating the effect of pom-znNS on serum uric acid, creatinine, ALT, and AST at 2nd and 4th week following infection are shown in (Table 2) The control positive (group 2) showed a remarkable increase in serum creatinine, uric acid, cholesterol, triglyceride, ALT, and AST compared with the control group and treated groups. Whereas, group (4) shows a remarkable reduction in serum creatinine and uric acid, and also a notable reduction in serum ALT, AST, cholesterol, and triglycerides in comparison with the control positive.

3.4 Antioxidant parameter activities

Table 3 shows the effect of PPE-ZNONps supplementation on antioxidant enzyme activities and MDA levels in the liver of broiler chicken. liver SOD activity in G4, treatment groups was higher than in control and G2 ($p < 0.05$). Also, serum CAT activity in G4 and G5 was higher than in the other groups ($p < 0.05$). The positive control group (G2), which was infected with *E. coli*, showed a significant reduction in GSH, SOD, and CAT activities compared to the control ($p < 0.05$). A significant increase in MDA was also reported.

3.5. Histopathological findings

Histopathological examination of liver tissues revealed significant differences among the five experimental groups. In the negative control group, the liver showed normal histoarchitecture with intact hepatic cords, central veins, and sinusoids, and no signs of inflammation or degeneration. In contrast, Group 2, the *E. coli*-infected untreated control, exhibited severe hepatic lesions including marked congestion of central veins and sinusoids, widespread hepatocellular necrosis, vacuolar degeneration, and perihepatitis indicated by heavy infiltration of mononuclear inflammatory cells, particularly in periportal regions. In Group 3, treated with 40 mg/L PPE-ZnO-NPs, moderate improvements were observed with reduced necrosis, mild to moderate congestion, and decreased inflammatory cell infiltration, though some vacuolar degeneration persisted. Group 4, receiving 80 mg/L PPE-ZnO-NPs, showed nearly normal liver morphology with well-organized hepatic cords, absence of necrosis and inflammation, and intact hepatocytes, suggesting a potent protective and restorative effect of the nanoparticles at this concentration. These findings confirm that PPE-ZnO-NPs exert dose-dependent hepatoprotective effects in *E. coli*-infected broilers, likely due to their antioxidant and anti-inflammatory properties. Finally, Group 5, treated with the highest dose of 120 mg/L PPE-ZnO-NPs, receiving 80 mg/L PPE-ZnO-NPs, demonstrated substantial histological improvement characterized by preserved hepatic architecture, mild and localized inflammatory infiltration, and minimal cellular degeneration, indicating effective hepatoprotection. (Fig. 3 liver A-E).

Table (2) Effect of pom-ZnO-NPs supplementation on serum biochemical parameters of broiler chicks.

Group	ALT (U/L)	AST (U/L)	Cholesterol (mg/dl)	TG (mg/dl)	Creatinine (mg/dl)	Urea (mg/Dl)
G1 21day	9.500± 0.55	12.50± 1.51	89.00± 6.0	100.5± 5.5	0.60± 0.02	15.50± 1.24
35 day	15.00± 1.04	17.50± 0.52	97.50±4.5	143.5±9.5	0.84± 0.04	37.00± 1.06
G2 21day	26.00± 3.09^{a}	29.50± 2.59^{a}	181.5± 2.5^{ab}	293.0± 17.0^{a}	0.92± 0.015^{a}	33.50± 2.52^{b}
35 day	35.50± 3.59^{a}	40.00± 3.08^{a}	253.0±11.0^{a}	303.5±12.5^{a}	1.35± 0.05^{a}	57.00± 2.08^{a}
G3 21 day	14.00± 1.04^{b}	17.50± 1.59^{ab}	137.0± 6.0^{b}	216.0± 4.0^{ab}	0.64± 0.025^{b}	25.00± 2.03^{b}
35 day	23.50± 2.53^{ab}	28.50± 2.53^{a}	219.0±1.0^{a}	227.0± 9.0^{ab}	0.99± 0.01^{a}	45.50± 1.54^{a}
G4 21 day	11.00± 1.06^{b}	15.50± 1.09^{b}	122.5± 3.5^{b}	189.5± 8.5^{b}	0.54± 0.06^{b}	21.00± 2.09^{b}
35	21.00± 2.01^{ab}	25.00± 2.01^{ab}	201.0±9.0^{a}	202.5± 5.5	0.86± 0.05^{ab}	43.00± 2.01^{ab}
G5 21day	20.00± 1.52^{ab}	24.50± 2.06^{ab}	144.5± 7.5^{ab}	258.5± 2.5^{a}	0.69± 0.03^{ab}	27.50± 1.53^{ab}
35 day	27.00± 2.03	31.50± 2.51^{ab}	216.5±1.0^{a}	279.0± 6.0^{a}	0.69± 0.03^{ab}	50.00± 1.04^{a}

Values are means ± SEM. a, b—Means in the same column with different superscripts are significantly different ($P < 0.05$). a: significant difference compared with the control group, b: significant difference compared with the infected group ($p < 0.05$).

Table (3) The Effects of pom-ZnO-NPs on antioxidant parameters in liver tissue

Parameter/groups	Group 1	Group2	Group3	Group4	Group5
MDA (nmol/gm)	104.8± 25.3	388.6±45.35^{a}	268.1± 62.95^{ab}	181.4± 71.06	299.2± 32.84
GSH (u/gm)	90.2± 10.7	33.35± 15.25^{a}	46.40 ± 14.4^{ab}	70.93± 11.47	51.43± 8.5
Catalase (u/gm)	1253± 24.59	696.8± 132.3^{a}	857.3± 151.8^{ab}	1170± 242.3	999.5± 168.6
SOD	1253±24.59	696.8± 132.3^{a}	857.3± 151.8^{ab}	1180± 242.3	999.5± 168.6

Effects of pom-ZnO-NPs supplementation on liver antioxidant parameters of broiler chicks. Values are means ± SEM. a, b—Means in the same column with different superscripts are significantly different ($P < 0.05$).

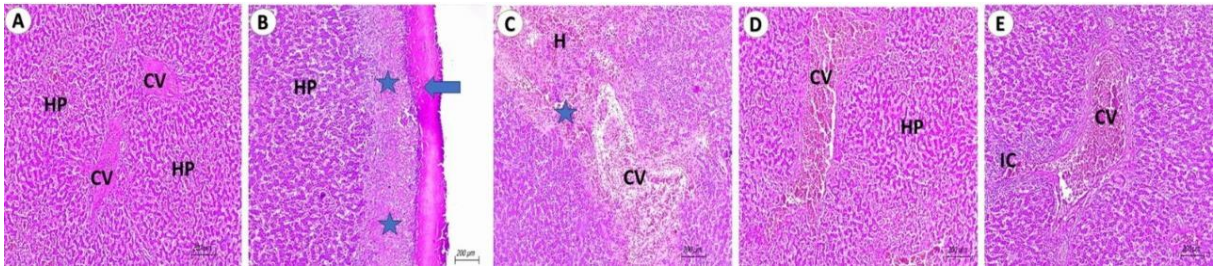

Fig. (3). Photomicrograph of the liver of 35-day-old chickens. A) Liver from the control group showing normal hepatic parenchyma (HP) around central veins (CV). B) Liver from the infected group showing perihepatitis (arrow) and the underlying area suffers from necrosis (stars). C) Liver from the 40 mg NZ group showing congestion (CV) and hemorrhages (H) with paracentral necrosis (star). D) Liver from the 80 mg NZ group showing congestion (CV). E) Liver from the 120 mg NZ group showing marked congestion (CV) and hemorrhages with infiltration of heterophilic inflammatory cells (IC). (H&E stain, scale bar 200 µm).

4. DISCUSSION

Avian pathogenic *Escherichia coli* is a significant threat to the poultry industry, causing colibacillosis, a bacterial illness that affects chickens and leads to significant economic losses. This disease manifests as a range of clinical signs, including respiratory distress, septicemia, and intestinal disorders, ultimately impacting growth performance, mortality rates, and flock uniformity. While *E. coli* is a normal inhabitant of the avian intestinal tract, APEC strains possess virulence factors that enable them to overcome host defenses and cause disease (Abdel-Hamed et al., 2025).

While *E. coli* primarily affects the intestinal tract in poultry, systemic infections, including those leading to colibacillosis, can cause liver and kidney damage, evidenced by elevated serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), also the most accurate markers for assessing kidney health and function are measurements of serum creatinine levels and urea (Wang et al., 2011). The liver enzymes, primarily localized within hepatocytes, are released into the bloodstream when these cells are damaged. The mechanism for liver and kidney damage in poultry with *E. coli* infection is often attributed to the inflammatory response triggered by bacterial endotoxins, such as lipopolysaccharide (LPS), found in the outer membrane of *E. coli*. Also, these endotoxins can trigger inflammation and damage to the kidney tissues, impairing their filtration capacity. Additionally, dehydration associated with *E. coli* infection can further stress the kidneys, exacerbating their dysfunction rather than direct invasion of the liver (Elbarbary et al., 2024a).

The increased levels of ALT, AST, urea, and creatinine observed in the present study are consistent with previous reports of liver and kidney damage in *E. coli* infections in poultry (Panth 2019; El-shenawy et al. 2023; Khairullah et al. 2024). Lipid profiles in broiler chickens infected with *Escherichia coli* can be significantly altered, reflecting the impact of inflammation, metabolic disruptions, and nutrient malabsorption. *E. coli* infection can lead to liver damage, impairing lipid metabolism and increasing circulating triglyceride levels. Additionally, intestinal damage caused by *E. coli* can lead to impaired fat absorption, potentially resulting in lower levels of circulating lipids. Changes in lipid profiles can also be influenced by alterations in the gut microbiome, which can be disrupted by *E. coli* infection Khairullah et al. (2024)**.** The significant elevation of lipid profiles observed in our study, particularly the increase in triglyceride and cholesterol levels, is consistent with previous studies that have documented similar changes in lipid metabolism during *E. coli* infections in poultry Wang (2020).

In the current study, the activities of liver enzymes, urea, creatinine, cholesterol, and triglycerides levels are significantly decreased in PPE-ZNONps, and the lower in these parameters are attributed to pomegranate extract and zinc nanoparticles (Elbarbary et al., 2024b). These findings are consistent with previous reports obtained by Rahmani and Aldebasi (2016), who proposed that dietary supplementation of PPE showed a potential hepatoprotective effect, depending on the presence of phenolic and antioxidant compounds in pomegranate extract, such as punicalagin, gallic acid, flavanones, and anthocyanidins, which were supported by the current blood metabolite results. Moreover, PPE has been shown to suppress pancreatic lipase activity, which lowers intestinal absorption of fat and excretes it in the form of stool (Kishawy et al., 2019). As per the current findings, serum creatinine and urea levels of broiler serum showed a significant decline in a dose-dependent manner when PPE-ZNONps were added to the challenge group, reflecting no toxicity on kidneys, but the control positive group reflected an increase in serum levels of urea and creatinine. This is because the nephrons' endothelium of the kidneys gets damaged due to toxic chemicals released by APEC. Mahmoud et al. (2020) unveiled an impressive decrease in the serum TG level with the dietary addition of 20 ppm ZnONPs. The present results are in concordance with Azam et al. (2019), who demonstrated that ZnONPs supplementation in a dose of 20 or 40 mg/kg diet reduced TG, cholesterol, and creatinine and increased HDL. Notwithstanding this, the results of Fathi (2016) showed that supplementing broiler feed with Nano-ZnO did not have any significant influence on the activators of ALT and AST and was related to elevated levels of serum cholesterol. *E. coli* infection in poultry triggers oxidative stress, which is induced by a mismatch between the production of reactive oxygen species (ROS) and the antioxidant defense system. It leads to lipid peroxidation, protein nitration, DNA damage, and apoptosis. It leads to the damage of the cell as well as to the pathogenesis of the disease. MDA is an indicator of oxidative damage and ROS-induced lipid peroxidation and is a measure of the degree of cellular damage (Zuo et al., 2014; Salah et al., 2025a). MDA typically rises with *E. coli* infection. Alternatively, the function of the antioxidant enzymes, including glutathione (GSH), catalase, and superoxide dismutase (SOD), may also decrease. Glutathione is a critical antioxidant that acts to neutralize free radicals, while catalase and SOD are major enzymes for detoxifying toxic ROS. Decreased levels of these antioxidants indicate a compromised antioxidant defense system and make the

birds more susceptible to oxidative damage (Khairullah et al. 2024).

The observed increase in MDA levels and the simultaneous decreases in GSH, catalase, and SOD in our study, consistent with previous research on E. coli infections in poultry, underscore the significant oxidative stress induced by the infection and the compromised antioxidant capacity of the birds (Fotouh et al., 2024b).

Based on our findings, the APEC infection considerably raised the broilers' MDA level (Table 2) in the untreated group, but adding pom-znoN to the groups that received it decreased MDA, prevented organism damage, and aided in the birds' recovery from Infection. Likewise, research has demonstrated that administering 1% pomegranate peel powder reduced the MDA content ($P < 0.05$) (Ahmadipour et al., 2021). Similarly, broiler antioxidant potential was increased by introducing Zn oxide nanoparticles. As demonstrated by a decrease in the liver's accumulation of malondialdehyde (MDA) and a rise in Cu/Zn SOD activity (Zhang et al., 2009).

According to Ahmadipour et al. (2021), antioxidant chemicals found in pomegranate extract can directly or indirectly reduce or block the oxidation of lipids in tissues. They either enhance the cell's inherent defensive mechanisms directly by scavenging for free radical species or indirectly by triggering the activities of antioxidant enzymes like SOD, CAT, etc. Two important liver antioxidant enzymes that catalytically scavenge free radicals and other ROS are superoxide dismutase (SOD) and catalase (CAT). This provides endogenous protection to biological systems. Pomegranate extract contains phenolic components that effectively eliminate free radical species and enhance the cell's other defense mechanisms. This, in turn, activates antioxidant enzymes like SOD and CAT within the body, thereby augmenting antioxidant defense. It has generally been proposed that hepatic antioxidant enzymes, such as SOD and CAT, are essential for protecting against oxidative stress because they catalytically scavenge reactive oxygen species (ROS) in tissue, enhancing endogenous health processes (Akuru et al., 2020). Likewise, research has demonstrated that administering 1% pomegranate peel powder reduced the MDA content ($P < 0.05$) (Ahmadipour et al., 2021). Similarly, the broiler antioxidant potential was increased by introducing Zn oxide nanoparticles. As demonstrated by a decrease in the liver's accumulation of malondialdehyde (MDA) and a rise in Cu/Zn SOD activity (Samy et al., 2015).

5. CONCLUSIONS

The addition of PPE-ZNONps to drinking water in chickens infected with *Escherichia coli* resulted in a considerable improvement in serum liver and kidney function indices, lipid profile, antioxidant defense capability, and liver damage, and this effect was dose-dependent. So it proposes performing more studies on this compound for usage as an alternative to antibiotics in the poultry sector.

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