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Efficacy of zinc nanoparticles against *E. coli* infection in broiler chickens

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ABSTRACT

Our study is intended to investigate the efficiency of the antibacterial activity of different materials such as antibiotic, organic zinc and zinc oxide nanoparticles (ZnO NPs) which experimentally tested against field avian pathogenic Escherichia coli (APEC) isolates. The study was carried out in a private broiler farm, 10 birds out of 60 (one-day-old-chicks) which were housed for 35 days were slaughtered and samples were taken from liver, lung and heart blood were positive for ***E. Coli***., these samples were cultured on special bacteriological media the remaining 50 birds were free from ***E. Coli.*** were divided into 5 groups 10 birds in every group, Group (1) free from *E. coli* infection and not treated kept as (negative control) Group (2) infected with isolated *E. coli* and not treated kept as (positive control). Group (3) infected with isolated *E. coli* and treated with difloxacin (10 mg/kg per day) on drinking water for 5 successive days for 3 times per day. Group (4) infected with isolated *E . coli* and treated with organic Zn (50 mg/kg) on drinking water for 5 successive days for 3 times per day. Group (5) infected with isolated *E . coli* and treated with ZnO NPs (100 mg/kg) on drinking water for 5 successive days for 3 times per day.

It was noticed that *E. coli* isolated from diseased broilers at an average incidence rate of 20%, recorded with highest resistant rate to erythrocin, gentamycin, tetracycline and amoxicillin (71.4, 57.1, 57.1 and 42.9 %, respectively) and highest sensitivity rate to difoxacin, amoxicillin and tetracycline (76, 28 and 23%, respectively) with minimal inhibitory (MIC), the birds treated with ZnO-NPs (G5) showed significant increase in Hemoglobin concentration, RBCs, PCV,WBCs count in ZONPs group comparing with control group. While, Serum cholesterol, triglyceride, low density lipoprotein, creatinine, uric acid and liver function test decreased Significantl, Also high density lipoprotein and innate immunity (IgA & IgM) were significantly increased in ZONPs group than control

In conclusion The therapeutic effect of ZnO Nano particles is recommended as an effective antimicrobial alternative against APEC strains.

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INTRODUCTION

The poultry industry has become the fastest growing industry and the biggest contributor to gross domestic product in many countries **(Youssef et al. 2022)**. The chicken is an example of efficient intensive animal agriculture and provides many valuable animal protein products **(Tizard et al. 2019).**

The main obstacle in the antimicrobial chemotherapy is increasing the resistance of antibiotics and chemotherapeutics, with the subsequence the insufficiency of antimicrobial administration **(Zchelz et al. 2006)**. In the past, antibiotics were widely used as growth promoters in animal feeds to enhance immune system and improve growth and production **(Vishwanathan et al. 2013)**. However, antimicrobial resistance and the possible transfer to human microbiota caused by the abuse of antibiotics have aroused global concerns **(Castanon 2007).** The demand is growing fast for dietary antibiotic alternatives to reduce the challenges in intensive animal and poultry production. In recent years, researchers have used from nanotechnology science and products as additives in broiler nutrition to achieve positive effects in poultry production, some of products are: nano-silver, nano-selenium and zinc oxide nanoparticles **(Ahmadi and Rahimi 2010)**.

Zinc (Zn) is an essential trace element , is an integral component of over 300 enzymes and is closely related to animal immune function and gut development **(Vondruskova et al. 2010)** .

Zinc is one of the essential elements that needed to growth and different physiological processes for poultry health and biological functions **(Maria Costa et al. 2023)**. Inorganic Zn, like Zn oxide was commonly used in poultry feed due to its low cost. However, the bioavailability of inorganic Zn is low, leading to a high level of Zn being added to the feed and resulting in other issues. Zinc oxide nanoparticles (ZnO NPs) have emerged as a promising alternative to their bulkier counterparts, but concerns about their safety persist. Zinc in nanoscale metal form has recently been suggested as a novel mineral feed additive in poultry diets **(Sizova et al. 2020)**. Zinc oxide nanoparticle (ZnO NP) shows high bioavailability, which is as bioavailable as the ionic zinc and can be used as an alternative to organic and inorganic zinc forms in animal feeds **(Sizova et al. 2020)**. ZnO NP also shows antibacterial, anti-inflammatory and antioxidant functions and has great potential for replacing antibiotics and traditional zinc sources, Zinc oxide nanoparticles (ZnO NPs) have involved a lot of consideration owing to their distinctive features. ZnO NPs can be described as particularly synthesized mineral salts via nanotechnology, varying in size from 1 to 100 nm, while zinc oxide (ZnO) is an inorganic substrate of zinc (Zn). Zn is a critical trace element necessary for various biological and physiological processes in the body. **(Rohit et al. 2023)**.

The aim of this study is to demonstrate that supplementation of Zn to broilers diet is important for support immune response and mineral retention and to state that zinc is a strong anti-inflammatory due to its roles in activation of natural killer cells and reducing reactive oxygen synthesis.

Also, current study was performed to investigate the efficiency of antibacterial activity of Zinic oxide nanoparticles compared to antibiotics and organic zinic against*E. Coli* (APEC) in broiler chickens.

MATERIALS and METHODS

Study Design :-

This study was carried out in a private broiler farm one-day old commercial Cobb chicks **(n = 60)** obtained from Al-Kahira poultry Company, 10th of Ramadan City, Sharkia Governrate, Egypt. Chicks were housed for 35 days under standard environmental and hygienic conditions and fed on a balanced commercial ration free from antibacterial agents and *water ad-libitum* before the beginning of the experiment to be sure that all chicks free from bacterial infection. So The cleaning and disinfection programs implemented at the farm under investigation received no particular emphasis, and the overall hygienic conditions on these farms were moderately fair.

Standard Samples collecting

Under aseptic conditions, one week old chicks (n = 10) were slaughtered then take samples from (liver, lung and heart blood) These samples were transferred on ice within 2 hours until they reached the laboratory **(cho et al. 2014)**. Following accurate identification, samples were sent immediately to the lab for microbiological analysis. Then cultured on special bacterial media and tested to ensure that they were free from any systemic *E. coli* infection.

Isolation and identification of pathogenic *E. coli*

Isolation of *E.coli* was applied according to **Cheesbrough, 2000.** The isolates of *E. coli* were streaked over Congo red agar and cultured for 72 h at 37 °C. Every sample pre-enriched in buffered peptone water then incubated for 24 h at 37°C in an aerobic environment **(Lee et al. 2008)**. Next step inoculum was inoculated with a loopful of Colonies to MacConkey's agar and Eosin Methylene Blue and incubated at 37 °C for 24 hours for identification, biochemical tests were used for *E. coli* confirmation. Selected metallic green colonies were sub-cultured on nutrient agar slopes then semisolid medium to be stored at 4 °C in preparation.

Antibiotic susceptibility test (Disk diffusion method)

Single disc diffusion method for detection of bacterial susceptibility to different antibiotics groups which obtained from Titanium trade pharmaceutical company as bottle contain 1 ml/L ready for use. The antibiotics used were gentamicin (CN), amoxicillin (AX), difloxacin (DIF), erythromycin (E), ciprofloxacin and tetracycyclin. According to the standard Kirby –Bauer disc diffusion method **(Quinn et al. 1994)** and results of inhibition zones` diameters translated into susceptible, intermediate and resistant categories according to **(CLSI, 2019).**

Preparation of experimental infective dose of Apathogenic E. coli (APEC O78):-

A strain (E. coli strain " E. coli ATCC 25922.") for challenge assay was previously isolated from liver samples with Primer sequence (5-3) F: GACCTCGGTTTAG-TTCACAGA-R CACACGCTGACGCTGAC-CA and subjected to molecular PCR with pure *E. coli* isolate was cultured on EMB and incubated aerobically at 37 C/24h. Colonies were picked up and inoculated on saline to obtain the stock inoculum for infection. The infective dose was enumerated to achieve 1×10^8 CFU/ml, and chickens were inoculated orally at two weeks old **(El-Boushy, 2006)**.

Preparation of metal nanoparticles

Normal zinc oxide was obtained from universal Laboratories U.F.C, packing under license of Belami Fine Chemicals Pvt. Ltd - ZnO nanoparticles (ZnO-NPs) were obtained from the Department of Nanotechnology, Faculty of Post Graduate Studies for Advanced Sciences, Benha University, Egypt. It is used in a powder form and average size (27.8 nm).

Experimental design

After two weeks old chicks the remaining chicks (n = 50), were reared and then observed daily for clinical signs, morbidity and mortality. Samples were collected for necropsy.

Table 1. Five groups of chicks were reared as the following :

No of Groups	Infection with *E . coli* (strain O78)	Treatment	
Group -1	(-)ve	Not Treated	Negative Control
Group -2	*(+)ve*	Not Treated	Positive Control
Group -3	*(+)ve*	Difloxacin 10 mg/kg.	
Group -4	*(+)ve*	Organic Zinc 50 mg/kg	
Group -5	*(+)ve*	Zno NPS 100 mg/kg	

N.B :
1- Every Group Contain 10 chicks .
2- Period of treatment : 3 times daily for 5 successive days .
3 – Route of treatment : per Os (Drinking Water) .

All groups treated (groups 3 , 4 & 5) were administered following the onset of clinical symptoms on the 20th old chicks and continued for five successive days via drinking water until the 24th day old and was adjusted from our in-vitro sensitivity tests, TEM, and cytotoxicity assays. The experiment was conducted until the 35th day of age. The animal studies were approved by Research Ethics Committee for environmental and clinical studies (Protocol number: 165,429) at Animal Health Research Institute (AHRI) and were carried out under Egyptian Ethics Committee Guidelines and the NIH guidelines for the Care and Use of Laboratory Animals. All animal experiments were performed following the arrive guidelines.

Post-mortem examination of dead broiler chicks

All birds were examined for classical signs of colibacillosis, enlarged spleen, pericarditis and perihepatitis. For each bird, up to 1g each of heart, kidney, liver, lung and spleen tissues were collected and sterile phosphate-buffered saline was added to each sample for homogenization using a Biomaster Micro-stomacher 80 (Seward, Worthing, West Sussex, UK) for 60 sec at high speed **(Kemmett et al. 2013)**.

Confirmation of Isolates by PCR

Briefly, 200 mL of the bacterial suspension in sterile normal saline was kept with 10 mL of proteinase K and 200 mL of lysis buffer at 56°C for 10 min. Post incubation, 200 mL of 100% ethanol was combined with the lysate. The specimen was rinsed and centrifuged following the manufacturer's guidelines. Nucleic acid was eluted with 100 mL of elution buffer supplied with the kits.

Blood Sampling, Hematological, and Biochemical Analysis

Blood samples were collected at the 7th, 15th day old chicks from the beginning of the treatment. The first blood sample was collected into heparinized tubes as an anticoagulant and used to determine hemoglobin concentration Hb%), packed cell volume percentage (PCV) **(Dacie and Lewis 1991),** red blood cells (RBCs), and white blood cells (WBCs) counts **(Toghyani et al. 2010)** while the second blood sample was collected from the wing vein into a sterile clean centrifuged tube without anticoagulant, left to coagulate at room temperature, and immediately centrifuged at 4000 rpm for 10 min to obtain serum. The obtained serum was transferred to dry sterile screw-capped tubes (Eppendorf) and stored in a deep freezer (− 20° C) to perform subsequent biochemical parameters as: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and uric acid using commercial kit. Activities of AST and ALT, Serum content of total proteins (TP), albumin (ALb) and serum globulin and alkaline phosphates (ALP) as indicators of liver functions . Meanwhile, Values of creat-

inine and uric acid were estimated as indicators for kidney functions and analyzed according to **(Fossati et al. 1980)** . All serum biochemical were measured spectrophotometric ally at wavelength 560 nm using commercial kits. Meanwhile, serum Immunoglobulin (IgA)**,** (IgG**)** and (IgM) levels were measured using test kits of Immunoassay IVD.

Statistical analysis:

Results were expressed as the mean ± standard error of mean. A *P*- value of less than 0.05 was considered significant **(Kinnear and Gray, 2006).**

RESULTS

1.Prevalence of *E.coli* isolates in the examined samples

The study focused on the prevalence of *E.coli* which isolated from visceral organs as (liver, heart and lungs) of clinically one day old commercial Cobb broiler chicks (n = 10) samples for every group revealed free from E.coli at the beginning of this study.

2.Isolation and identification of APEC

Table 2. G (2): infected with *E . Coli* & not treated, G (3):infected with *E . Coli* & treated with difloxacin ,G (4): infected with *E . Coli* & treated with organic Zn,G(5): infected with *E . Coli* & treated with ZnO NPs (*E.coli* isolates on EMB agar with green color with metallic sheen)

Groups	*No of (E.coli) (+) Samples*
2	8
3	4
4	6
5	3

N.B : Number of birds were 10 in every group .

3. Biochemical identification of E. coli

Table 3. Biochemical reaction of MacConkey's positive E. coli isolates

Biochemical test	**Reaction**
Indole test	Positive
Methyl red test	Positive
Voges-Proskauer test (VP)	Negative
Citrate utilization test	Negative
Urease test	Negative
Triple sugar iron test (H2S production) test	Negative
Catalase test	Positive
Oxidase test	Negative
Sugar fermentation test	Positive
Nitrate Reduction test	Positive

4. Disk diffusion method

In order to study the effect of different antibiotics agent which examined by disc diffusion test under the standard conditions as mentioned before in the methods. A total 21 isolates were tested against: gentamicin, amoxicillin , difloxacin, erythromycin, ciprofloxacin and tetracyclin

Table 4. Overall antimicrobial susceptibility patterns of E. coli isolated

Antimicrobials	Resistant N (%)	Intermediate N (%)	Sensitive N (%)
Erythromycin	15 (71.4)	4 (19)	2 (9.5)
Gentamicin	12 (57.1)	6 (28.5)	3 (14.3)
Ciprofloxacin	5 (23.8)	2 (9.5)	1 (4.76)
Amoxicillin	9 (42.9)	6 (28.5)	6 (28.5)
Difloxacin	4 (19)	1 (4.76)	16 (76)
Tetracycline	12 (57.1)	4 (19)	5 (23.8)

N.B : Number of Isolates were 21*E.Coli* Isolates .

5. Post-mortem examination of dead broiler chicks

Post-mortem examination of broiler chicks with colibacillosis appear as (accumulation of fluid around the heart &discoloured liver and Pericarditis (fibrin-based lesions around the periardium).

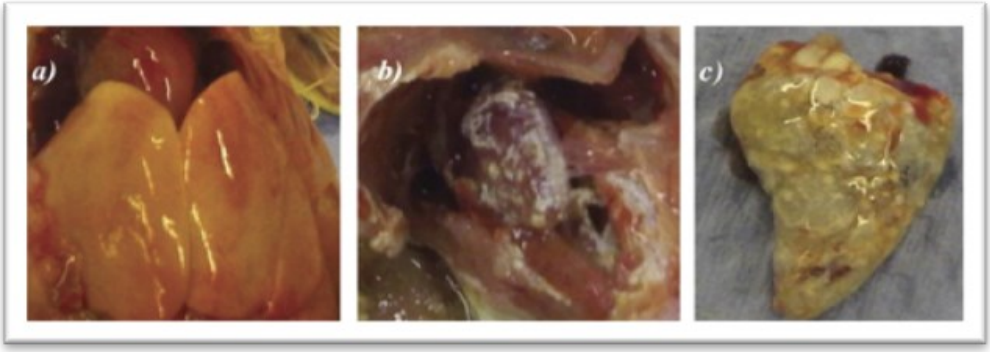

Fig (1):Post-mortem examination of broiler chicks

6. Confirmation of Bacterial Isolates by PCR

PCR was applied to confirm the results of conventional isolation through targeting the relevant species conserved genes as demonstrated in Figures (4) as all *E. coli* isolates were tested using a 16S rRNA primer with a band size of "585pb." The positive control was *E.coli* ATCC 25922 .

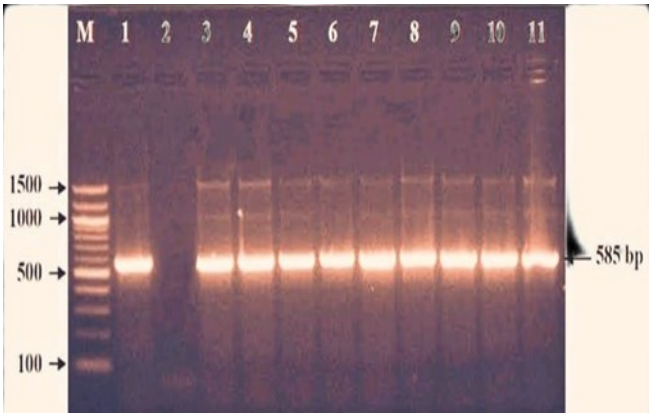

Fig (2): 1.5% EB stained agarose gel amplification from *E. coli* isolates, where L 100 bp DNA ladder; (2) is negative control; (1) P is positive control "E. coli ATCC 25922."

Hematological parameters changes:

Results of our experiment showed that, treated chicks with ZnO NP diet had significantly enhancement of RBC count, Hb concentration and PCV % while WBCs count revealed significant decrease in all treatments and compared with infected groups and increased when compared with control group .

Table 5. Mean values of hematological parameters in chicks before and after treatment by Organic Zn, Difloxgard and ZnO NP throughout the experimental period

Groups / Parameters	Healthy control chickens	Infected chickens with *E. coli* before treatment	Diseased chicks after treatment with		
			Organic Zn	Difloxgard	ZnO NP
Hb gm/dl	11.64± 0.359 ^{c}	10.88± 0.317 ^{d}	12.20± 0.38 ^{ab}	12.39± 0.382 $^{a b}$	12.56± 0.391 ^{a}
RBCs x10^{6}/µl	3.71± 0.231 $^{a b}$	2.85± 0.177 ^{c}	3.19± 0.199 $^{a b}$	3.65± 0.228 $^{a b}$	4.31± 0.268 ^{a}
PCV %	36.4± 1.25 ^{c}	35.65± 1.23 ^{d}	38.2± 1.32 ^{c}	39.03± 1.35 ^{ab}	39.76± 1.37 ^{a}
WBCs x10^{3} /µl	14.40± 0.679 ^{d}	17.21± 0.821 ^{a}	15.23± 0.727 ^{c}	16.44± 0.785 ^{b}	16.83±0.803 ^{b}

9.Serum biochemical analysis:

Table 6. Mean values of liver function tests in chicks before and after treatment by Organic Zn, Difloxgard and ZnONP throughout the experimental period:

Groups / Parameters	Healthy control chickens	Infected chicks with *E. coli* before treatment	Diseased chicks after treatment with		
			Organic Zn	Difloxgard	ZnO NP
AST u/l	24.12± 1.244 ^{d}	26.77± 1.381 ^{a}	26.05± 1.34 $^{a b}$	25.48± 1.31 ^{b}	25.09± 1.295 $^{b c}$
ALT u/l	29.384± 1.231 ^{e}	32.92± 1.38 ^{a}	32.03± 1.34 ^{ab}	31.27± 1.31 ^{c}	30.52± 1.28 ^{d}
T.P. g/dl	3.92± 0.148 ^{e}	6.46± 0.247 ^{a}	5.68± 0.22 ^{b}	5.03± 0.19 $^{b c}$	4.78± 0.18 ^{d}
ALP g/dl	165.39± 1.519 ^{e}	174.40± 1.60 ^{a}	173.31± 1.592 ^{b}	172.40± 1.58 $^{b c}$	169.22± 1.55 ^{d}
Albumin g/dl	2.061± 0.061 ^{a}	1.04± 0.031 ^{e}	1.11± 0.033 ^{d}	1.32± 0.039 ^{c}	1.51± 0.045 ^{b}
Globulin g/dl	1.859± 0.221 ^{e}	5.42± 0.644 ^{a}	4.57± 0.543 ^{b}	3.71± 0.441 ^{c}	3.27± 0.389 $^{c d}$
A/G ratio	1.109± 0.049 ^{a}	0.191± 0.008 ^{e}	0.243± 0.010 $^{c d}$	0.356± 0.016 ^{c}	0.462± 0.020 ^{b}

Table 7. Mean values of lipid profile , serum creatinine and uric acid tests in chicks before and after treatment by Organic Zn, Difloxgard and ZnO NP throughout the experimental period:

Groups / Parameters	Healthy control chicks	Infected chicks with *E. coli* before treatment	Diseased chicks after treatment with		
			Organic Zn	Difloxgard	ZnO NP
Cholestrol (mg/dl)	135.27± 13.81 [d]	149.69± 15.28 [a]	146.34± 14.94 [a b]	143.21±14.62 [b]	140.06± 14.30 [b c]
Triglycerides (mg/dl)	56.01±5.50 [d]	78.22±7.68 [a]	75.44±7.41 [a b]	70.44±6.92 [b]	66.32±6.51 [c]
HDL (mg/dl)	87.15± 4.23 [e]	89.76±4.36 [c d]	91.43± 4.44 [bc]	96.54± 4.68 [b]	101.32± 4.92 [a]
LDL mg/dl	46.54± 4.09 [a]	44.64± 3.92 [b]	39.82± 3.50 [b c]	32.58± 2.86 [d]	25.48± 2.24 [e]
VLDL mg/dl	21.34± 3.24 [a]	15.64± 2.37 [b]	15.09± 2.29 [b c]	14.09± 2.14 [c d]	13.26± 2.01 [e]
Creatinine (mg/dl)	0.771± 0.026 [e]	2.87± 0.097 [a]	2.29± 0.077 [b]	1.84± 0.062 [c]	1.70± 0.057 [c d]
Uric acid (mg/dl)	7.12± 0.250 [e]	10.63± 0.373 [a]	9.74± 0.342 [b]	9.09± 0.312 [b c]	8.52± 0.299 [d]

10.Immunological changes:

Table 8. Mean values of IgA, IgG and IgG tests in chicks before and after treatment by Organic Zn, Difloxgard and ZnONP throughout the experimental period

Groups / Parameters	Healthy control chickens	Infected chickens with *E. coli* before treatment	Diseased chicks after treatment with		
			Organic Zn	Difloxgard	ZnO NP
IgA µg/mL	115.90±14.29 [b]	253.41± 14.88 [a]	230.12± 13.51 [c]	217.92± 12.79 [d]	188.21± 11.50 [e]
IgM µg/mL	1.98± 0.104 [e]	4.41± 0.227 [a]	4.12± 0.212 [a b]	3.72± 0.192 [c]	2.86± 0.147 [d]

DISCUSSION

Zink nanoparticles have proven to be effective therapeutic agents due to their antimicrobial activity and it has great potential for replacing antibodies.

The current study was performed to investigate the efficiency of antibacterial activity of Zinic oxide nanoparticles compared to antibiotics and organic zinic against*E. Coli* (APEC) in broiler chickens.

Table (2) revealed that *E.coli* (APEC) isolates collected from internal organs (liver lung and heart) were 8, 4, 6 and 3 (out of 10) for groups 2,3, 4 and 5 respectively, this agrees with **El-Seedy et al. 2019** who reported that a highly prevalence rate of *E.coli* isolates from liver and heart while **Hasan et al. 2020** stated that highly prevalence rate from pericarditis.

In table (3) biochemical test proved that isolates from all groups (2,3,4 and 5) were *E.coli* (APEC) this agrees with **(Quinn et al. 1994).**

In table (4) the highest drug resistance was 89.2 % for Erythromycin, then 86.6%, 72.5%, 13.3 %, 7.5% and 3.3% for Amoxycillin, Tetracycline, Gentamycin, Ciprofloxacin and Difloxacin.

This agrees with the findings of previous studies **(Orrett and shurl, 2001)** which

showed that antibiotics as amoxicillin and tetracycline showed more than 45% resistance. on the other hand, the resistance rates recorded in this study are higher than the results of **Khan et al. (2002)** as the incidence of *E. coli* was reported to be high in urine samples and females more susceptible than males on the other hand it was lower than the results of **Okonko et al. 2009.**Also High level of resistance of *E. coli* was reported to tetracycline from a study conducted in Ethiopia (**Andargachew., 2006)** and to erythromycin (**Scheutz et al. 2004).**

In the current study, all clinical samples of *E. coli* showed high resistance rates to erythromycin and amoxicillin (**Bharathi et al. 2008)** as the prevalence of amoxicillin resistance was very high with MIC (96%), but amoxicillin resistance was lower with MIC (30%). Moreover, **Felix et al. 2013** confirm that *E. coli* is highly resistance to commonly used antibiotics that lead to treatment failure.

On the other hand, *E. coli* isolates were sensitive to gentamicin, ciprofloxacin and Chloramphenicol. Similar studies (**Tesfaye et al. 2009)** revealed that high sensitivity to ciprofloxacin and gentamicin have been recorded from previous studies conducted in Nigeria and India **Bharathi et al. 2002**. And furthermore, validation utilizing PCR-based procedures, a specific, rapid, precise, and reliable method for detecting and characterizing as little as 100 *E. coli* bacteria, is critical for validating the data acquired from serological approaches **Fanjip et al. 2022**. as in this study, the DNA of E. coli isolates was amplified by PCR using ECO-f and ECO-r primers with the goal of detecting a 585 bp amplicon of the E. coli 16S rRNA gene **Tonu et al. 2011**. Recent technologies are being widely applied to poultry sector like nanotechnology **(Nabi et al. 2020).** ZnO-NPs have a dose-dependent effect on bird performance and physiological state of poultry and livestock **Mahmoud et al. 2021.** Like other metal oxides, ZnO-NPs exhibit antibacterial activity against a wide variety of bacteria. This study demonstrated that ZnO-NPs could exhibit potent anti-E. coli activities. As an outcome of the use of ZnO-NPs, in vivo efficacy was demonstarted through a reduction of cumulative mortalities in either group infected or treated with ZnO-NPs. The evidence indicates that ZnO-NPs are highly effective antibacterial agents which similar to the work of **Brayner et al. (2006)** who demonstrated that ZnO-NPs stop the growth of bacteria like *E. coli* during the interaction between ZnO-NPs and microbes and Zn2+ ions are released through channels, destroying bacterial viability. Zinc is the second most abundant and crucial nutritional trace element found in the body (**Wan and Zhang 2022).** Also Zn is important for several biological processes, like growth, metabolism, reproduction and wound healing (**Lee et al. 2009).** Zn requirement for poultry is 40 ppm like NRC, 1994. However, on commercial levels, feed manufacturers supplement their feed with an additional 100–120 ppm of Zn to speed up the growth of their chicks **Feng et al. 2010.** In addition to our result. **Zhao et al. (2014)** study revealed a higher inclusion levels of Zn which may affect the balance of other microelements and reduce the stability of vitamins and other nutrients and increase its accumulation inside the animal body. Nanotechnology has been widespread their particles having a size of less than 100 nm in three dimensions (**Titma et al. 2016)** which increased bioavailability, digestibility due to the particle's minor size and increased surface area to volume ratio (**El-Dawy et al. 2023).** Moreover, NPs are competent enough to pass through the GIT and distribute themselves further into the blood stream and intended organs. The infectious microorganism is the most serious threat to the poultry industry. Antibiotics were widely used to control infectious microorganisms to improve growth and productivity.

However, the prolonged use of antibiotics resulted in antimicrobial resistance and the possibility of transmission to humans, raising global concerns (**Yusof et al. 2023).** ZnO NP exhibits anti-bacterial activity, which demonstrated bactericidal action in contradiction of both gram-negative/positive bacteria , However, they are capable of producing toxicity in birds, which generally depends on the size, shape, and NPs concentration along with the route of exposure (**Arabi et al. 2004)** which reported that several new approaches have been applied to control microbial infec-tion, such as metal oxide nanoparticles (MONPs), a new class of materials for potential use in scientific research and health-related applications. Indeed, nanotechnol-ogy can be used in the pharmaceutical industry, animal health, veterinary medicine, and various extents for ani-mal production, hence **Fawzia et al. (2023)** showed that it considered an effective, eco-friendly, and low-cost strategy for disease control.

The liver is a vital organ that is widely studied when investigating the effects of feed supplementation since it serves as the primary storage site for minerals and vitamins (**Chen et al. 2006)** as confirmed that it regulates their distribution where Zinc is a part of liver enzymes as cofactor including alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and aspartate aminotransferase (AST); as well it is typically found in large quantities in liver enzymes. Enlarged liver is a symptom of an underlying problem that is associated with the inflammation of the organ in the infected chickens with *E. coli*, whereas Serum Total protein, globulin, albumin / globulin ratio, aspartate aminotransferase (AST), (ALT) and ALP Were significantly higher in infected when compared with control. Whereas, **Yusof et al. (2023)** indicated that albumin revealed significant decrease in infected group with *E. coli* when compared with control. Meanwhile, in the treated group with ZnO NPs there was improvement in liver function which more significance when compared with groups treated with organic Zn and difloxacin due to strong bactericidal activity of ZnO NPs against poultry-relevant foodborne pathogens including *Salmonella* spp., *E. coli,* and *S. aureus* . This finding was consistent with that demonstrated the potential antibacterial activity of ZnO NPs, which could potentially replace conventional antibiotics in poultry production. However, the major concern of using ZnO NPs as an antibacterial agent in poultry is their bactericidal effect on commensal bacteria in the gut, particularly, beneficial bacteria such as lactic acid bacteria this results agree with **Abd El-Hack et al. (2021).** also The effectiveness of NPs in preventing the development of an extensive range of disease causing agents (**Saravanan et al. 2018)** which showed that it could potentially offer an alternative to antibiotics. **Samy et al. (2022)** Suggesting that the use of ZnO NPs can be safe without disrupting the balance of commensal bacteria. Overall, dietary ZnO NPs at doses of 70 and 100 mg kg − 1 diet was found to be beneficial in terms of antimicrobial efficacy.

Serum Cholesterol, TG and LDL Were significantly higher in infected group with *E. coli* when compared with control. Whereas, HDL showed revealed significant decrease in infected group with *E. coli* when compared with control group these results agreed with **Syama et al. (2013)** who attributed this to Liver tissue exposed to zinc nanoparticles showed significant increased in lipid peroxides formation and induce oxidative stress and per oxidative membrane lipids cells of birds that fed diet including ZONPs .Whereas in contrast with **Malcoln -calis et al. (2000)** as they reported that serum cholesterol concentrations were not altered by added zinc in different concentrations. Measurements of serum uric acid and creatinine concentrations are the most sensitive indicators to estimate kidney state and functions. Our re-

sults revealed that adding ZONPs to broiler diets resulted in significant reductions in serum uric acid and creatinine concentration in treated group with ZnONP when compared with infected these results are in harmony with the recent results by **Abdel-Wareth et al. (2022)** due to the role of higher bioavailability zinc in the form of nanoparticles. Also The results of IgA , IgG and IgM results revealed significant decrease in all treated groups when compared with diseased group before treatment in comparison with control group. These results in harmony with, H-S. **Sundar & Kim, (2005)** who demonstrated that supplementation of Zn to broilers diet was important for support immune response and mineral retention and similar to **Dreno et al. (1992)** which indicated that zinc is a strong anti-inflammatory property because of its roles in activation of natural killer cells and reducing reactive oxygen species synthesis.

CONCLUSION

It was noticed that *E. coli* isolated from diseased broilers recorded with highest resistant rate to colistin, difloxacin, gentamicin and erythromycin with and highest sensitivity rate to amoxicillin, doxycycline and gentamicin with minimal inhibitory (MIC) the birds treated with ZnO-NPs (G5) significant increase in Hemoglobin concentration, RBCs, PCV, WBCs count in ZONPs group comparing with control group . While, Serum cholesterol, triglyceride, low density lipoprotein, creatinine, uric acid and liver function test decreased Significantly, while high density lipoprotein and innate immunity (IgA & IgM) were significantly increased in ZONPs group than control

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