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EVALUATION OF EFFICACY COMBINATION OF ZNO NANOPARTICLES AND RESISTED ANTIBIOTICS AGAINST AVIAN PATHOGENIC *E.COLI*

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ABSTRACT

The antibiotic resistance of Avian Pathogenic *E. coli* is considered one of the biggest public health concerns worldwide and with the growing necessity to find unconventional approaches for formulating new forms of safe and price of effective antibiotics for monitoring the spread of resisted pathogens globally, zinc oxide (ZnO) has the prospective to influence many aspects because of their antimicrobial efficacy. Therefore, our study is intended to evaluate the *in vivo* antibacterial activity of the chemically synthesized PEG-6000 coated-ZnO nanoparticles in low (11.6mg/ml) and high (23mg/ml) doses alone and in combination with florfincol and streptomycin, experimentally. The obtained results showed that oral administration of a low dose of ZnO NPs (11.6 mg/ml) alone or with florfincol and streptomycin revealed mild efficacy against APEC infection, while a high dose of ZnO-NPs (23 mg/ml) whether alone or combined with florfincol and streptomycin for 3 days of treatment, gave good improvement, that was confirmed grossly, and by histopathological inspection of the liver and kidney. It could be concluded that not only do PEG-6000 Coated ZnO NPs have a powerful antibacterial effect against APEC in broilers, but they also can enhance the antibacterial activity of resisted antibiotics.

Keywords: APEC, ZnO Nanoparticles, Histopathology, Broiler

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INTRODUCTION

Escherichia coli normally inhabitant in the poultry intestine, but under stress factors certain strains, became virulent, those called avian pathogenic *E.coli* (APEC), spread into the different internal organs, causing the systemic fatal disease colibacillosis (McPeake *et al*., 2005). APEC is the main reason for economic losses as a result of morbidity, mortality, costs attained in prevention and disease control, and the condemnation of poultry carcasses worldwide (Ronco *et al*., 2015).

Colibacillosis denotes two-thirds of the reported bacterial infections in poultry production (Souillard *et al*., 2011), and mainly affects birds 4-8 weeks of age, though adults can be affected either by primary or secondary infection (Rashid *et al*., 2013), causing several local and systemic infections like septicemia, omphalitis, swollen head syndrome, cellulitis, pericarditis, and perihepatitis (Paixao *et al*., 2016). The most predominant serogroups related to colibacillosis are O1, O2, and O78, and their incidence differs between farms and countries (Mehat *et al*., 2021).

Several approaches were performed for controlling APEC infections, such as hygiene improvement, competitive exclusion of probiotics usage, vaccination, and the introduction of immune-potentiates; however, each of the following had limited achievement (La Ragione *et al*., 2004). This has necessitated the use of antimicrobial chemotherapy to decrease the incidence and mortality of outbreaks of avian colibacillosis (Dheilly *et al*., 2012). However, *E. coli*, like many other bacteria, can create antibiotic resistance and modern reports have described increased resistance to those antimicrobial agents commonly used for treatment (Yang *et al*., 2004). With the running out of options to treat bacterial diseases, the appearance of nanoparticles has emerged as a novel antimicrobial. ZnO NPs have several advantages: high antibacterial effectiveness at low concentrations against *E.coli* (Zhang *et al*., 2010), minimal toxicity, and decreased side effects (Lara *et al*., 2010).

The antibacterial activity of the ZnO NPs is induced by the release of Zn2+, which has a significant influence on the active transport inhibition, amino acid metabolism, enzyme system distribution and the liberation of oxygen species from the surface of ZnO (OH-, H2O2, and O2-2), causing fatal damage to microorganisms, indicating that ROS formation is the key mechanism for antibacterial ZnO NPs activity (Sirelkhatim *et al*., 2015).

Water molecules' presence around ZnO NPs motivates ZnO-Zn bond formation between nanoparticles, leading to agglomerate formation (flocculation), that hinders the dispersibility and use of ZnO NPs (Yıldırım and Durucan, 2010). Thus the PEGylation process is used to mend the stability of ZnO NPs against precipitation through the prevention of sticking the particles together (Liufu *et al*., 2004). Our study was considered to investigate the *in vivo* efficacy of two different concentrations of PEG-6000 Coated ZnO NPs and the synergistic effect of resisted florfincol, and streptomycin with ZnO NPs.

MATERIALS AND METHODS

PEG-6000 Coated ZnO Nanoparticles

In situ PEG-6000 Coated ZnO nanoparticles, with sizes of 20 and 50 nm

were supplemented by the Avian and Rabbit Medicine Department, Assiut University, Assiut, Egypt (Aml *et al*., 2023). ZnO NPs physically were spherical shape by TEM; white powder; stable colloid in a mixture of water, ethanol, and PEG-6000, an absorption peak seen at ~ 432 cm^{-1} optical properties (Abs.) of λmax = 301 nm and 380 nm and average size (TEM) in the range of 19-67 nm. The intense absorption peaks at ~ 1006, 1362, 1473, and 2877 cm^{-1} were observed by IR spectra, approving the presence of polymer molecules covering ZnO nanoparticles. ZnO powder was used, ovened for 3 hours at 160 ˚C, then dissolved in distilled water, to avoid particle aggregation and deposition. The sample was vortexed (10 minutes) and then sonicated (90 minutes) and the obtained suspensions (100 mL with a 1M ZnO concentration), were considered as a stock solution and then to be diluted for chicken treatment usage.

Experimental Chicks

One hundred one-day-old commercial broiler chicks (Ross128), obtained from a local Egyptian poultry company, reared in experimental area of the Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Assiut University, approved by the National Ethical Committee of the Faculty of Veterinary Medicine, Assiut Egypt, according to the OIE standards for use of animals in research following ARRIVE guidelines under No. 06/2023/0094, subject to the following ordinary vaccination program; Hitchner B1 (7 days of age) against Newcastle disease and IBD vaccine (12 days old). Chicks were raised in a routine management practice and an optimum hygienic environment.

***E. coli* strain**

The *E. coli* strain serotype O_2, resistant to florfenicol and streptomycin, was previously isolated from broiler chickens, suffering from high mortalities, serotyped, and used for challenges (Badry, 2018). The bacterial suspension was adjusted to contain 10^8 CFU/mL by the colony count technique.

Experiment Design

Chicks were divided into ten equal groups (10 chicks per each) in separated small floor pens, and at 15 days old, they were challenged with *E. coli* strain serotype O_2, (0.1ml) of the inoculum containing 10^8cfu/ml, by intra-tracheal route, except group (10) served as the negative control (not challenged, not treated). Chickens were observed daily for signs, mortalities, and postmortem lesions. After 27 hours from infection, chickens were treated with two different doses of PEG-6000 coated ZnO nanoparticles, by oral gavage, as shown in Table (1), in addition to the resisted antibacterial drugs florfincol and streptomycin (Sigma-Aldrich) in drinking water. All groups were examined clinically after the third- and fifth-days post-treatments (dpt).

Table 1: Experimental design

Group No.	Treatment used and Dose(mg/kg b.w)
G1	Low dose of PEG-6000 Coated - ZnO NPs (11.6mg).
G2	High dose of PEG-6000 Coated- ZnO NPs (23mg).
G3	Low dose of PEG-6000 Coated - ZnO NPs Nanoparticles (11.6mg)+ florfincol(30mg)
G4	High dose of PEG-6000 Coated - ZnO NPs (23 mg) + florfincol(30mg)
G5	Low dose of PEG-6000 Coated- ZnO NPs (11.6mg) + streptomycin (40mg)
G6	High dose of PEG-6000 Coated- ZnO NPs (23 mg) + streptomycin (40mg)
G7	florfincol(30mg)
G8	streptomycin (40mg)
G9	Infected , not treated (control + ve)
G10	Not infected , not treated (control –ve)

APEC recovery from treated groups was done by using an *E. coli* count on EMB agar. The livers of chicks were removed aseptically and homogenized. The homogenates were tenfold serially diluted before plating on the EMB, according to (Ahmed *et al*., 2020). The EMB agar plates were incubated overnight at 37 ˚C, and the *E.coli* colonies were counted.

Histopathological examination

Specimens from the liver and kidney from groups 1 and 2 after 5 days post-oral treatment were sampled, fixed in 10% neutral buffered formalin, dehydrated in graded alcohol series, cleared with xylene, and embedded in paraffin wax. Sections of 4 μm were cut and stained with hematoxylin and eosin (H&E) (Bancroft *et al*., 1996), inspected by a light microscope, and photographed by a digital camera.

RESULTS

Clinical signs

All infected treated groups suffered from mild clinical signs of colibacillosis compared with chicks in the positive control group (group 9), which exhibited typical signs of colibacillosis after 3 days from infection including symptoms of weakness, depression, loss of appetite, dyspnea, coughing, sneezing, gasping, and nasal discharge. Control negative (group 10), chicks remained active throughout the experimental period as they appeared healthy without any clinical signs. The morbidity percentage was 100%, while there were no mortalities in all infected groups.

Post mortem lesions

In the control positive group (G 9), congestion in all organs with pericarditis, fibrinous perihepatitis (Fig.1B), splenomegaly, air sacculitis, pneumonia, and enteritis were the most observed common lesions. The severity of the lesion increased and persisted until the end of the experiment.

Group 7 and group 8 which were treated only with florfincol and streptomycin, respectively showed mild congested liver, mild air sacculitis, congested lung, and enteritis. Group 5 was treated with PEG-6000 coated ZnO NPs (low dose 11.6 mg/kg) and streptomycin showed mildly congested liver, nephritis, and highly congested lung, and there was a minor improvement in the p/m picture after the fifth day of treatment.

Group 3, which was treated with a low dose of PEG-6000 Coated ZnO NPs (11.6 mg/ kg) and florfincol showed a closely normal carcass appearance after 3 days post-treatment, but there was mild congestion in the liver after 5 days from treatment. Group 1 which was treated only with a low dose of PEG-6000 Coated ZnO-NPs (11.6 mg/ kg), showed normal carcass appearance with mild congested liver and pericarditis after 3- and 5-days post-treatment.

Each of group 2, which was treated with 23mg/kg PEG-6000 coated ZnO NPs (high dose), group 4 (23mg/ kg ZnO NPs + florfincol) (Fig.2A) and group 6 (23mg/ kg ZnO NPs + streptomycin), after 3 days from treatment showed a normal appearance. After 5 days of treatment in group 2 (Fig.2C), there was enlarged congested kidney, bursal exudate, and hemorrhagic spots in the thigh muscle and congested liver in group 3 (Fig.2B), Group 10, (control negative) (Fig.1A) showed a normal carcass appearance.

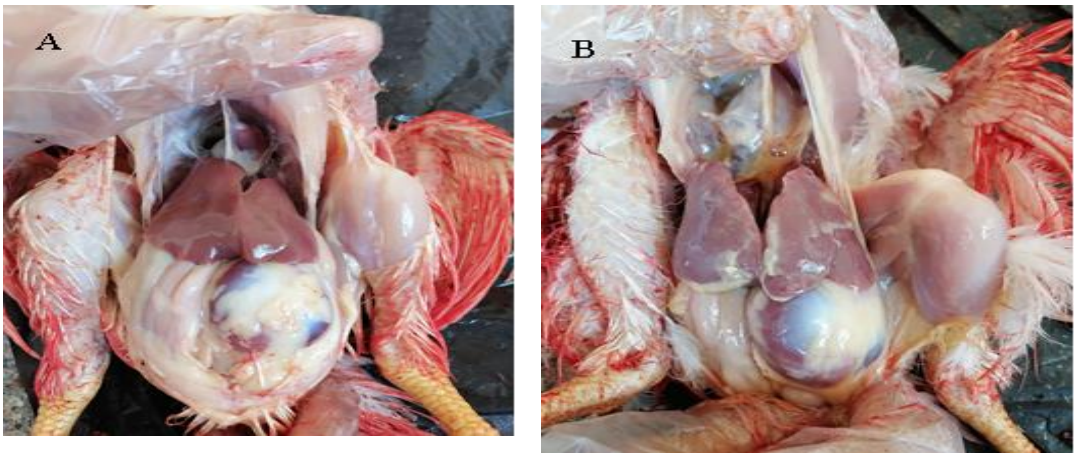

Fig. 1: (A) 23-day-old chicks from the negative control group displaying normal post-mortem picture. **(B)** 23-day-old chicks from the positive control group showed cloudiness and thickness of the air sac accompanied by serous exudates, marked pericarditis, and fibrinous perihepatitis.

Fig. 2: (A) 23 days old chicks treated with PEG-6000 Coated ZnO-NPs high dose+ florfincol after 3 days showing normal appearance. **(B)** 23-day-old chicks treated with low dose PEG-6000 Coated ZnO-NPs +florfincol after 5 days showing mild congested liver**. (C)** 23 days old chick treated with 23mg/ kg.bw PEG-6000 Coated-ZnO-NPs after 5 days showed an enlarged congested kidney.

APEC recovery from experimental groups by *E. coli* counts on (EMB) agar:

Positive control (group 9) illustrated the highest re-isolation rate while the rate of the negative control group was zero. All infected treated groups showed variable rates of *E. coli* re-isolation after 3 days of treatment and although the persistence of *E. coli* was observed in groups 1, 2, 7, and 8, there was complete inhibition of *E. coli* growth in groups 3, 4, 5, and 6 after 5 days of treatment (table 1).

Table 1: APEC recovery rate from slaughtered chicks after each treatment

G. No.	Treated group	Post 3 days	Post 5 days
G1	Low dose of PEG-6000 Coated ZnO NPs (11.6mg).	$3x10^4$	$1X10^4$
G2	High dose of PEG-6000 Coated ZnO NPs (23mg).	$1x10^5$	$3X10^4$
G3	Low dose of PEG-6000 Coated ZnO NPs (11.6mg) + florfincol	$1x10^5$	0
G4	High dose of PEG-6000 Coated ZnO NPs (23 mg) + florfincol	$1x10^5$	0
G5	Low dose of PEG-6000 Coated ZnO NPs (11.6mg) + streptomycin	$10x10^5$	0
G6	High dose of PEG-6000 Coated ZnO NPs (23 mg) + streptomycin	$8x10^4$	0
G7	florfincol	$4x10^6$	$3X10^5$
G8	streptomycin	$3x10^7$	$3x10^6$
G9	(control + ve)	$30x10^6$	$50x10^6$
G10	(control –ve)	0	0

Histopathology

The score of histopathological lesions in the liver and kidneys to detect the residual effect of PEG-6000 Coated ZnO NPs in low and high doses are summarized in Table (2). The lesions were as follows:

Liver:

The liver of control-negative chicks (G10) revealed normal hepatic architecture (Fig 3. A). Examination of the liver from chicks infected with *E. coli* (control + ve G9) revealed a severe inflammatory reaction that was noticed mainly in the periportal area and consisted of severe congestion, severe periportal infiltration of heterophils, necrosis of the epithelial lining bile ductules, an interstitial inflammatory reaction, and multiple areas of mononuclear cellular infiltration. Damage to the endothelial lining of blood vessels and thrombosis can also be seen (Fig.3.B-E). The liver of chicks treated with PEG-6000 Coated ZnO NPs at dose of (11.6 mg/kg) G1 showed no infiltration of heterophils. Congestion and necrosis of the epithelial lining bile ductules were still present. There were sporadic areas of mononuclear cellular infiltration (Fig.3.F-H**).** Administration of PEG-6000 Coated ZnO NPs at dose of (23 mg/kg) G2 for 3days showed great improvement, in which there was minor periportal mononuclear cellular infiltration and normal bile ductules, no necrosis of the bile ductular epithelium, and no heterophilic infiltration **(**Fig.3 I-J). Through the examination of the liver of chicks treated with PEG-6000 Coated

ZnO NPs at a dose of 23 mg/kg G2 for 5 days showed congestion of the blood vessels and sinusoids, hemorrhage, and sporadic areas of mononuclear cellular infiltration (Fig.3 K-L).

Kidneys:

The kidneys of control chicks (G10) showed normal architecture (Fig.4.A). Kidneys of birds infected with *E. coli* (control + ve G9) showed a layer of hemorrhage and fibrinous inflammation surrounding the kidneys, congestion, hemorrhage, a focal area of mononuclear cellular infiltration, and coagulative necrosis of the renal epithelium. Coagulative necrosis was manifested by increased eosinophilia of the cytoplasm and pyknosis of the nuclei. The epithelial lining was desquamated in some renal tubules (Fig.4.B-D). Kidneys of chicks administrated PEG-6000 coated ZnO NPs (11.6 mg/kg) revealed focal areas of mononuclear cellular infiltration and swelling of some renal tubular epithelium. Few renal tubules showed desquamation of the lining tubular epithelium (Fig.4.E-F). PEG-6000 Coated ZnO NPs (23 mg/kg) for 3 days revealed normal kidney architecture (Fig.4.G). Examination of kidneys from birds treated with PEG-6000 Coated ZnO NPs at a dose of 23 mg/kg for 5 days showed severe congestion of the blood vessels, hemorrhage, and coagulative necrosis of renal tubules (Fig.4 H-I).

Table 2: Histopathological lesion score in liver and kidneys in PEG-6000 Coated ZnO-NPs low and high doses groups after 5 days post-treatment compared with control groups.

Groups Lesions	Control -ve	Control +ve	ZnO NPs low dose (5dpt)	ZnO NPs high dose (3dpt)	ZnO NPs high dose (5dpt)
Liver					
-Congestion	0	3	3	1	3
-Hemorrhage	0	3	3	-	3
-Focal area of heterophilic	0	3	-	-	-
infiltration	0	3	3	1	2
-Mononuclear cellular	0	3	3	-	-
infiltration					
-Necrosis of the epithelial lining of bile ductules					
Kidneys					
-Congestion	0	3	2	0	3
-Hemorrhage	0	3	0	0	3
-Coagulative necrosis of	0	3	2	0	3
renal tubules	0	3	3	0	3
-Mononuclear cellular infiltration					

Lesion score: 0: no lesions, 1: mild, 2: moderate, 3: severe

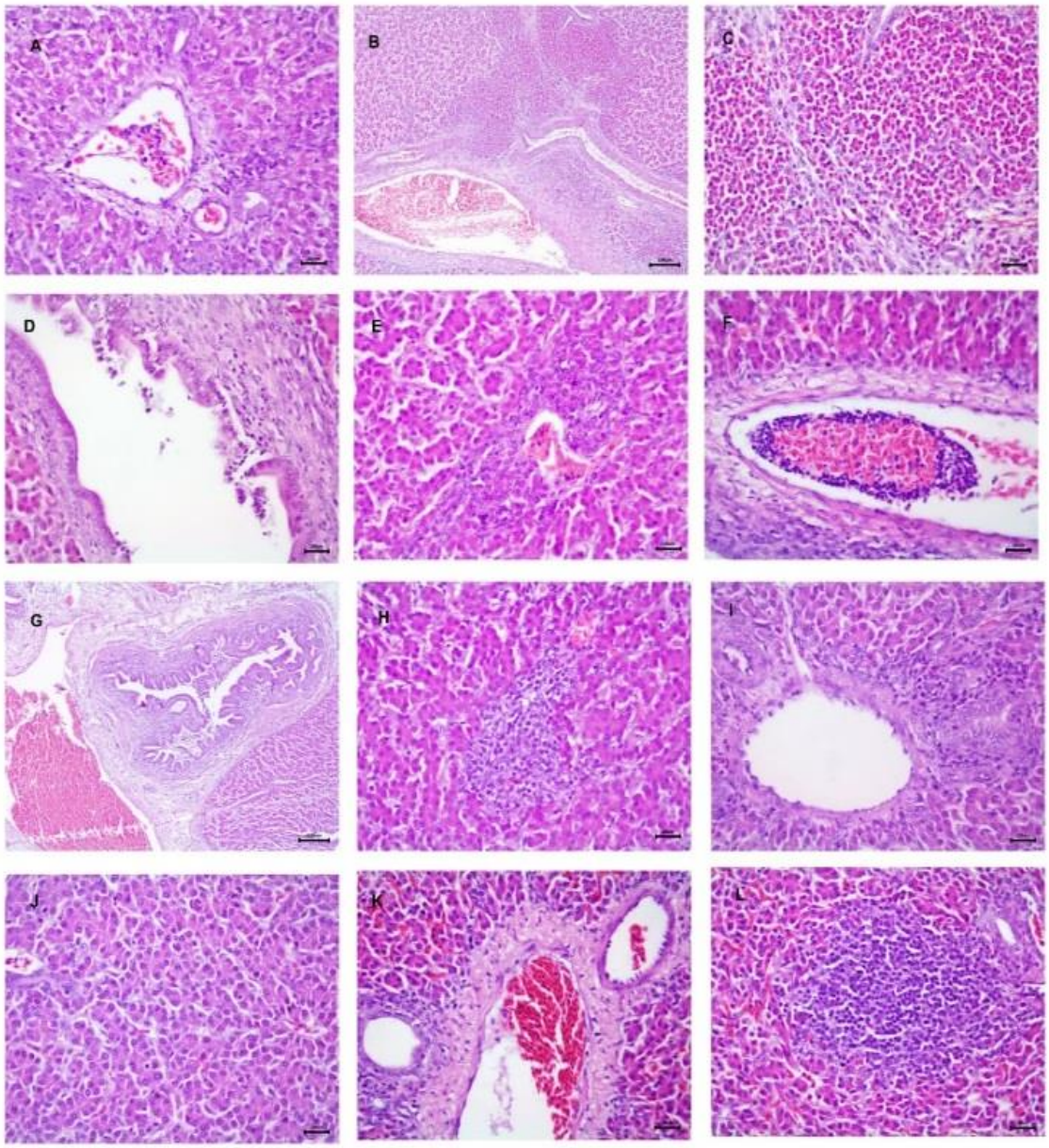

Fig. 3: Photomicrograph of liver tissues (A) Control negative group, liver showing normal hepatic architecture. (B-F) Liver of bird infected with *E. coli.* (B) Severe periportal heterophilic infiltration and congestion. (C) Higher power showing infiltration heterophils. (D) Necrosis of the bile ductular epithelium. (E) Interstitial infiltration of inflammatory cells and focal mononuclear cellular infiltration. (F) Thrombosis of blood vessels. (G-H) Liver of birds treated with ZnO NPs low dose. (G) Portal area showing congestion of blood vessels and necrosis of bile ductular epithelium. (H) Interstitial infiltration of inflammatory cells and focal mononuclear cellular infiltration.(I-J) Liver of bird-treated ZnO NPs high dose for 3 days. (I) Portal area with mild mononuclear inflammatory reaction and normal bile ductules. (J) Normal hepatic architecture. (K-L) liver of the bird treated with ZnO NPs for 5 days. (K) Congestion and dilatation of sinusoids. (L) Mononuclear cellular infiltration. HE

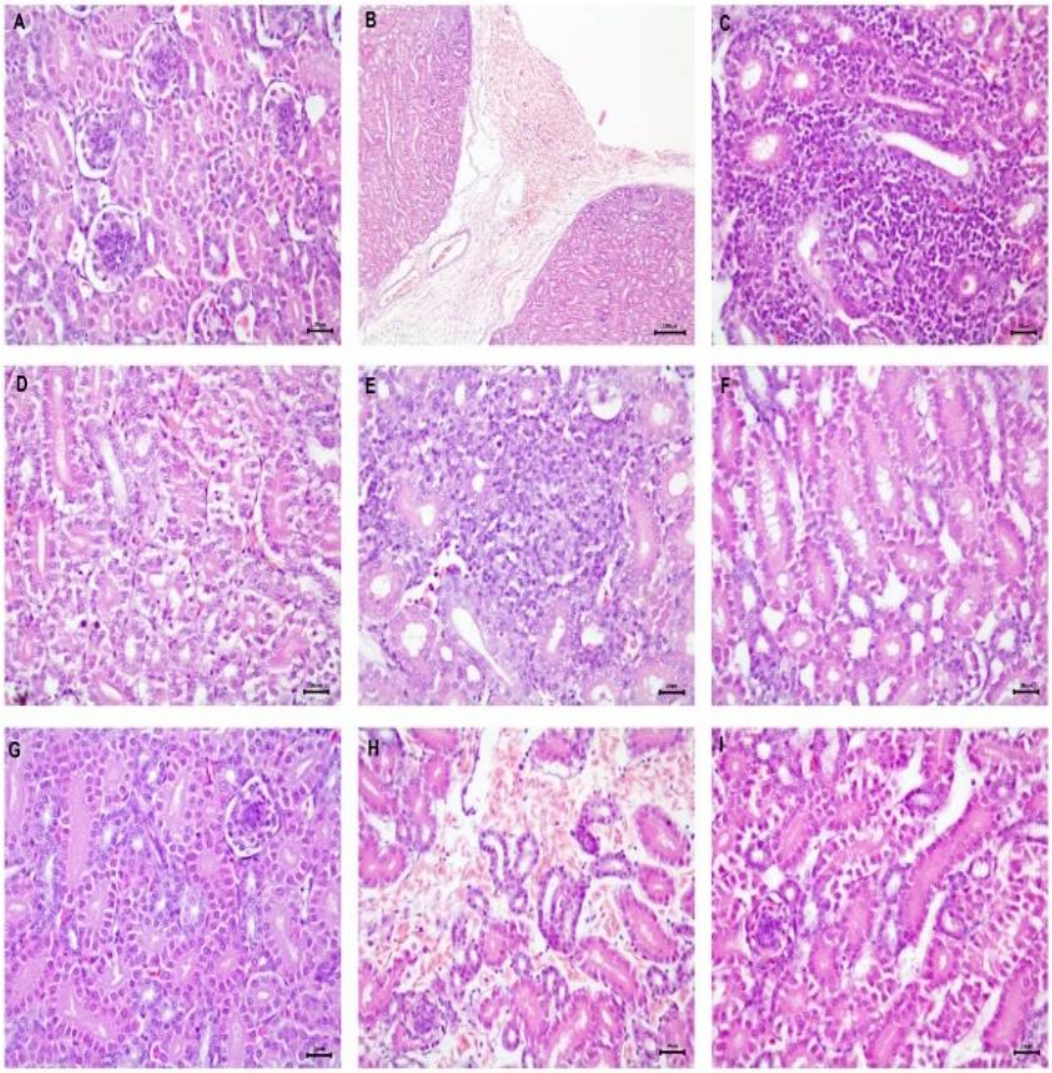

Fig. 4: Photomicrograph of kidney tissues (A) Control negative group, kidney showing normal architecture. (B-D) kidney of a bird infected with *E. coli*. (B) Showing layers of hemorrhage and fibrinous inflammation surrounding the kidneys and invading the interstitial tissue. (C) Focal mononuclear cellular infiltration (D) Necrosis of the renal tubular epithelium manifested by increased eosinophilia of cytoplasm and pyknosis of the nucleus. (E-F) Kidney of birds treated with ZnO-NPs low dose. (E) Mononuclear cellular infiltration. (F) Swelling of renal tubular epithelium. (G) Kidney of bird-treated ZnO-NPs high dose for 3 days showing normal kidney architecture. (H-I) The kidney of the bird was treated with ZnO NPs high dose for 5 days. (H) Hemorrhage within the interstitial tissue. (I) Necrosis and desquamation of the renal tubular epithelium by H&E.

DISCUSSION

APEC strain is regarded as a sub-pathotype of extra-intestinal pathogenic *E. coli* (ExPEC) causing avian colibacillosis, which is considered a potential zoonotic agent and some of these strains cause severe human diseases such as hemorrhagic colitis and hemolytic uremic syndrome (Nolan *et al*., 2013). The pathogenicity of *E. coli* strain O2, in the control positive group by intratracheal inoculation revealed depression, closed eyes and respiratory symptoms (gasping, rales, and nasal discharge), these findings in agreement with (Barnes, 1994). All infected treated groups suffered from mild clinical

signs of colibacillosis. The control negative group remained active throughout the experimental period.

At necroscopy, congestion was the most prominent picture in all organs combined with perihepatitis, pericarditis, air sacculitis, pneumonia, and splenomegaly which comes in line with that obtained by (Sharada and Ruban, 2010), who observed the fibrinous perihepatitis and pericarditis in broiler chickens infected with *E.coli*. All groups treated with PEG-6000 Coated-ZnO-NPs in high dose (23mg/kg b.w) alone or with florfincol and streptomycin after three days from treatment showed normal clinical pictures, while after 5 days of treatment; enlarged congested kidney, bursal exudate and congested liver were observed. There were different rates of re-isolated *E. coli* in all challenged treated groups after 3 days of treatment but after 5 days of treatment, *E. coli* growth was completely inhibited in groups that were treated with the two different concentrations of PEG-6000 Coated ZnO NPs with florfincol and streptomycin, showing the effective synergism between ZnO NPs and the resisted antibiotics.

The pathological lesions observed in the liver of birds infected with *E. coli* were congestion, hemorrhage, severe periportal infiltration of heterophils, focal areas of mononuclear cellular infiltration, and necrosis of the epithelial lining of bile ductules. Similar results were described before by (El-Ghany and Madian, 2011; Abalaka *et al*., 2017). Kidney lesions in birds infected with *E coli* include hemorrhage, congestion, coagulative necrosis of renal tubular epithelium, and mononuclear cellular infiltration, (Dutta *et al*., 2013; Abalaka *et al*., 2017) described similar lesions. PEG-6000 Coated ZnO NPs 23mg/kg.bw for 3 days treatment induces great improvement in both kidneys and liver. This improvement may be attributed to that ZnO NPs possess large surface areas with small sizes that enable NPs to exhibit effective antibacterial activity through production of reactive oxygen species (ROS) generation (Guo *et al*., 2015), the loss of bacterial cellular integrity (Brayner *et al*., 2006), and may due to the PEGylation process, which has been proven to reduce the cytotoxicity of ZnO NPs effectively, while ZnO NPs at dose of 23mg/ml for 5 days showed congestion of blood vessels and sinusoids, hemorrhage and sporadic areas of mononuclear cellular infiltration. Similar lesions were observed in the liver of rats after oral administration of ZnO NPs (11 mg/kg) for five consecutive days (Ben-Slama *et al.*, 2015). It was detected that oral administration of ZnO NPs can cause degenerative changes, necrosis, focal leukocytic infiltration of the liver, minimal renal lesions, hyalinosis, and lymphocytic myocarditis in the heart. It has been claimed that ZnO NPs cause hepato-and nephrotoxicity through epigenetic changes in the gene expression of mtTFA, and PGC-1α that may subsequently cause mitochondrial dysfunction which activates the generation of ROS and oxidative stress (Radi *et al*., 2021). Also, the toxic effects of ZnO NPs may be attributed to their solubility, resulting in increased intracellular Zn2+, as nanoparticles were predicted to increase the occurrence of inflammatory reactions in different organs, especially the lymph nodes (Mokhtar *et al*., 2019).

CONCLUSION

Exploring the *in vivo* antibacterial potential of ZnO NPs by oral treatment against APEC exposed a very good clinical prognosis. The best treatment for APEC was achieved using PEG-6000 coated ZnO NPs (23mg/kg) for 3 days alone or synergistically with both resisted florfincol and streptomycin, enhancing their efficacy, which was confirmed by gross examination, reisolation, and histopathological inspection. Continuous usage of the same dose for 5 days led to internal binging of histopathological toxicity appearing in the liver and kidney, so it was important to pay attention to the dose of ZnO-NPs to avoid toxicity. Further effective combination regimens using ZnO NPs, and resistive antibiotics are recommended for treating multi-drug resistant APEC.

Abbreviations: avian pathogenic E. coli: APEC; EMB: Eosin Methylene blue; hematoxylin and Eosin: H&E

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Declarations

Ethics approval and consent to participate

All methods were approved by the Ethical Committee of Veterinary Medicine Faculty, Assiut University, Assiut, Egypt (Protocol number 06/2023/0094) according to The standards of OIE for the use of animals in research in accordance with the relevant guidelines and regulations.

تقييم الفعالية المركبة لجزيئات أكسيد الزنك النانوية والمضادات الحيوية المقاومة ضد بكتيريا الإشريكية القولونية الممرضة للطيور

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تعد مقاومة بكتيريا الإشريكية القولونية للمضادات الحيوية واحدة من أكبر مخاوف الصحة العامة في جميع أنحاء العالم، ومع تزايد الضرورة لإيجاد طرق بديلة لصياغة أنواع جديدة من المضادات الحيوية الآمنة والفعالة من حيث التكلفة للسيطرة على انتشار مسببات الأمراض المقاومة على مستوى العالم، تتميز الجسيمات النانوية لأكسيد الزنك (ZnO) بالقدرة على التأثير على العديد من الجوانب بسبب فعاليتها المضادة للميكروبات. لذلك، تهدف هذه الدراسة إلى تقييم النشاط المضاد للبكتيريا للجسيمات النانوية لأكسيد الزنك المطلية بـ PEG-6000 المصنعة كيميائيًا بجرعات منخفضة (١١,٦ ملجم / مل) وعالية (٢٣ ملجم / مل) بمفردها وبالاشتراك مع الفلورفينكول والستربتومايسين، تجريبيًا في دجاج التسمين.

أظهرت النتائج التي تم الحصول عليها أن تناول جرعة منخفضة من الجسيمات النانوية لأكسيد الزنك (11.6 ملغم / مل) عن طريق الفم بمفرده أو مع الفلورفينكول والستربتومايسين ذات فعالية خفيفة ضد عدوى الإشريكية القولونية الممرضة للطيور ، في حين أن الجرعة العالية منه(23 ملغم / مل) سواء بمفرده أو بالاشتراك مع الفلورفينكول والستربتوميسين لمدة ٣ أيام من العلاج اظهرت فعالية جيدة جدا وتم تأكيده بالفحص النسيجي للكبد والكلى.

ومنه يمكن القول أن الجسيمات النانوية لأكسيد الزنك المطلي بـ PEG-6000 ليس فقط له تأثير ضد عدوي الايكولاي في دجاج التسمين، ولكن أيضًا لديه القدرة على تعزيز النشاط للمضادات الحيوية المقاومة ضد البكتيريا.