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ORIGINAL ARTICLE

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Overcoming Nephrotoxicity of Oral and Injectable Colistin through Niosomal Nano Formula Drug Delivery against Avian Pathogenic *E. coli* in Broiler Chicks

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Abstract
Colibacillosis is an infectious disease produced by avian pathogenic *E.coli* associated with reduced productivity, high mortality and condemnation rates as well as increased treatment costs. Colistin has become the predominant treatment option against multidrug resistant (MDR) Gram-negative bacteria. Nevertheless, progression of renal damage caused by injection of colistin frequently hinders the attainment of ideal therapeutic dosages. The present work aimed to formulate colistin-loaded niosomes (CLN) to improve efficacy and decrease toxicity of colistin as a potential treatment against avian pathogenic *E.coli* in broiler chicks. The CLN in tween 60, cholesterol and dihexadecyl phosphate in the molar ratio of 1:2:0.1 was chosen as an efficient carrier for delivering colistin. The minimum inhibitory concentration for CLN was approximately 12 times lower than that of free colistin with enhanced pharmacokinetic parameter, demonstrating the higher efficacy of CLN. Efficacy and safety of CLN were investigated *in vivo* using an experimental bird model using *E. coli*. In contrast to control positive group, serum albumin, total protein and creatinine concentrations were significantly lower following parenteral CLN administration. Histopathological examination of the kidney demonstrated that CLN inhibits nephrotoxic effects when compared to free colistin. Additionally, microscopical examination of liver, lung, and heart samples demonstrated the safety of the CLN. In contrast to colistin sulphate, niosomal colistin demonstrated superior pharmacological activity and efficacy suggesting that administering parenteral CLN is more effective and safer than conventional colistin.

Keywords
Broilers, Colistin, *E.coli,* Nephrotoxicity, Niosomes

1. Introduction
Broiler chickens aging 4-6 weeks are susceptible to colibacillosis, which is caused by avian pathogenic *E. coli* (APEC) **(Vandemaele et al., 2002).** The disease is typified by acute lethal septicemia or sub-acute fibrinous pericarditis, airsacculitis, salpingitis, and peritonitis **(Alexander and Senne, 2008)**. Colibacillosis is economically significant in poultry industry **(Lutful Kabir, 2010)**. Colistin is a polypeptide antibiotic that is used orally in veterinary medicine to treat or prevent enteritis **(EMEA, 2002)** especially those caused by *E. coli* and *Salmonella spp*. **(Collell and Segura, 2013)**. Colistin acts by altering the bacterial cell membrane's permeability. Electrostatic interactions among the cationic polypeptide and anionic molecules of lipopolysaccharide (LPS) of Gram negative bacteria's outer membrane promote bacterial cell membrane derangement; this type of interaction is an irreversible binding associated with bactericidal activities. Following this process, the cell envelope becomes more permeable, allowing contents to leak out and, eventually, cell death **(FAO, 2006; Coria et al., 2011)**. Furthermore, colistin exhibited strong anti-endotoxin activity; Gram negative bacteria endotoxin is the lipid A portion of LPS molecules and colistin attaches to and neutralizes LPS. However, colistin toxicity is reported because of its strong interaction as a cationic drug with highly anionic nerves in the body such as renal nerves, cochlear and that's of skeletal muscles nerves. Permanent or irreversible binding causes nephrotoxicity and neurotoxicity **(Lim et al., 2010)**. Hence, to overcome these problems, colistin-loaded niosomes has been developed.

Niosomes are a sort of nanoparticles that include cholesterol and nonionic surfactants. To enhance the delivery of medications that are water-soluble, like colistin, niosomes are being considered as a potential pharmacokinetic system **(Manosroi et al., 2003; Bnyan et al., 2018)**. The selectivity, efficiency, and bioavailability of drug are all enhanced by niosomes **(Manosroi et al., 2003; Nowroozi et al., 2018)**. Researchers have demonstrated that niosomes enhance target-site uptake, prolong circulation time, decrease toxicity and strength stability of medication **(Manosroi et al., 2003; Nowroozi et al., 2018)**. To ensure that the generated niosomes have a very negative charge, the charge inducer dihexadecyl phosphate was used **(Waddad et al., 2013; Bnyan et al., 2018)**. The present work aimed to formulate a parenteral colistin-loaded niosomes (CLN) to improve the efficacy and decrease the toxicity of colistin as a potential treatment against multi-drug resistant avian pathogenic *E.coli* in broiler chicks.

2. Materials and Methods

All animal handling and care were done in accordance to Beni-Suef University's Institutional Animal Care and Use Committee (IACUC) ethical guidelines for treating animals with approval number 022-504).

2.1. *In vitro* Characterization and Preparation of Colistin-Loaded Niosomes

The thin film hydration method was utilized to formulate colistin-loaded niosomes (CLN) **(Kazi et al., 2010).** Using of differential scanning calorimetry (DSC) (60F3, Maia, Germany) CLN's thermal behavior compatibility with its individual components was investigated **(Gamal et al., 2020)**. CLN were examined using transmission electron microscopy, morphology and surface characteristics of **(Gamal et al., 2021)**. A carbon-coated copper grid was treated with CLN sample, which was subsequently stained using phosphotungstic dye. Polydispersity index (PDI) and size are significant characteristics of niosomes that govern distribution, particles' dispersion and homogeneity **(Nowroozi et al., 2018)**. By determining its zeta potential, the electrostatic charge, surface characteristics, and stability of the CLN were assessed. **(Chaw and Kim, 2013; Bnyan et al., 2018)**. In three separate experiments, the zeta ziser instrument (Malvern, Germany) was applied to measure the PDI, particle size and zeta potential through dilution of CLN (1mL) with 9mL of distilled water **(Gamal et al., 2021)**.

2.2. Serum Concentrations Study

The study involved two groups, each consisting of 5 broiler chickens aging 30 day of weight 1.9±0.05kg. Chickens in group 1(G1) and group 2(G2) were given a single oral dose of 4mg/kg colistin sulphate and niosomal colistin respectively. Blood samples (approximately 1mL) were gathered from the chickens after treatment at various time intervals: 0, 0.5, 1, 2, 4, 6, 8, 12, and 24hrs. After clotting at room temperature for 2h, the samples of serum were separated by centrifugation then stored at -20°C in plastic tubes until analysis using the HPLC method from the US Pharmacopeia, with a 212nm wavelength and a 4.6-mm ×25-cm × 5µm column at a flow rate of 1mL/min. The mobile phase included a mixture of 0.1 M tri basic sodium phosphate and acetonitrile in a 77:23 ratio, with the pH adjusted to 3. The samples were analyzed using an Agilent 1200 HPLC system. **(United States Pharmacopoeia 36 Monographs Monographs for Colistimethate Sodium and Colistin Sulfate, 2019)**

2.3. Field Isolates of *E. coli*

Two field strains of *E. coli* were phenotypically and genpty pically identified in the previous study **(Salam et al., 2024)** as congo red binding positive, serum resistant, MDR, colistin sensitive and harbor the following virulence associated genes (*iss*, *tsh*, *fim*H *and iroN*).

2.4. Assessment of Pathogenicity in One Day-Old Chicks

To ascertain the pathogenicity of field isolates *E. coli*, forty (one-day-old) chicks were obtained from a commercial source. *E. coli* strains were re-cultivated in tryptone soya broth at 37°C for 24h. bacterial culture containing about $3x10^8$ colony-forming units/mL (CFU/mL) were prepared. At 5 day old, the chicks were randomly divided into 4 equal groups and then inoculated with 1mL and 1.5mL for subcutaneous injection and oral route, respectively. Chicks were monitored every day for ten days according to **(Vidotto et al., 1990; El-Sawah et al., 2018).**

2.5 Assessment of Minimum Inhibitory Concentration (MIC) as an *in-vitro* Efficacy Indicator

MIC was measured using the microplate dilution method on 96 well (U-shaped) plates. *E. coli* inoculums were standardized to give density $1.5x10^8$ CFU/mL according to **(Elisha et al., 2017)**. MIC of four antimicrobials [niosomal colistin (from 0.0195 to 120µg/mL), colistin, cefotaxime and ciprofloxacin (from 0.0097 to 60µg/mL for each antibiotic)] against selected *E. coli* strain was determined according to **Yu et al. (2004).**

2.6 Chick's Challenge In-vivo Assay

The aim of the experiment was to evaluate the efficacy of colistin and colistin-loaded niosomes in six-day-old chicks that were previously infected with *E. coli* (colistin-sensitive strain). The challenged dose was 0.5 ml of the bacterial suspensions adjusted to $3x10^8$ CFU/mL and administrated parentally at the 5th day of age according to **Fernandez et al., (2002).** Ninety (one-day-old) broiler chicks were acquired from a commercial hatchery. They were housed in cages with a high level of biosecurity and watered ad Libitum and fed on a typical commercial ration devoid of antibiotics. Colistin (G3, G5) and colistin-loaded niosomes (CLN) (G4, G6) were given with dose 80000IU/Kg. body weight (2.7mg/kg) and administered by two routes orally (given 4 doses for 4 successive days) and parentrally (given two doses day after day via subcutaneous injection), respectively. The administration was immediately after the appearance of clinical signs on chicks post artificial infection. Six equal groupings of chicks were created as follow: G1 retained as a negative control group (untreated, non-infected), G2 was experimentally infected with *E. coli* strain as positive control. The vaccination programs against ND, IB and IBD viruses were applied. The chicks were observed daily throughout the experiment (2 weeks). Clinical signs were recorded and PM examinations was conducted on any dead chicks as well as three euthanized chicks.

2.6.1. Samples Collection and Analysis

Blood was drawn from the wing vein and placed in heparinized and non-heparinized micro hematocrit tubes. Plasma and serum were separated and tested for creatinine and urea according to **Pandya et al. (2016)** and albumin, globulin, and total protein according to **Tothova et al. (2019)**.

2.6.2. Histopathological Examination

Tissue Samples (kidneys, liver, heart and lungs) were collected from freshly dead or euthanized birds (at the end of the experiment). Samples were preserved in formalin 10%, then subjected to paraffin embedding technique. Following mounting, sections underwent hematoxylin and eosin (H&E) staining **(Bancroft and Gamble, 2008).**

2.7. Statistical Analysis

The SPSS software, version 22.0, Inc., Chicago, IL, USA, was used for statistical analysis.

3. Results

3.1. *In vitro* Preparation and Characterization of Colistin-Loaded Niosomes

Thermograms of colistin, optimum CLN, cholesterol, and dihexadecyl phosphate (DDP) were shown in **Fig. (1).** Sharp endothermic peaks were visible on the DSC curves of cholesterol, DDP, and colistin at 217°C, 25°C, 79°C, and 149°C respectively, which corresponded to their melting points. The morphology of the CLN, showing spherical distributed vesicular structures were shown in **Fig. (2).** PDI, zeta potential and particle size of the optimum CLN were shown in **Fig. (3).**

3.2. HPLC Method Validation and Chromatograms

As shown in **Fig. (4)** and **Table (1),** the concentrations were 0.48µg/mL for the free colistin and 0.78µg/mL for the CLN at 0.5h demonstrated that CLN enhanced bioavailability of colistin compared to free colistin**.** After oral administration of two different formulations, the peaks of colistin were observed **(Fig 5).**

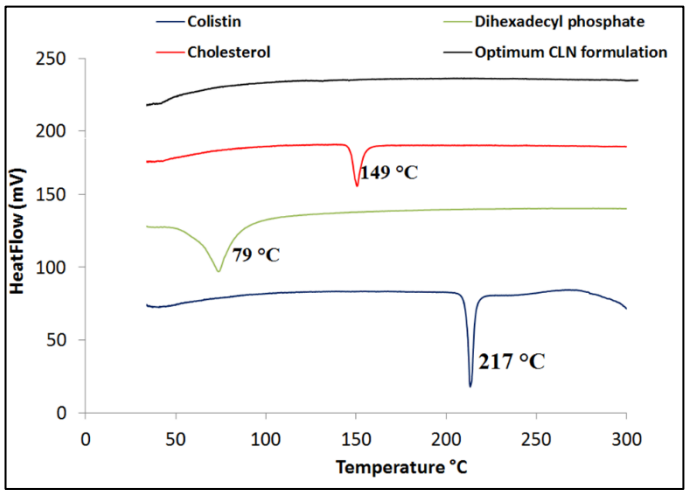

Fig. 1. Differential scanning calorimetry of CLN

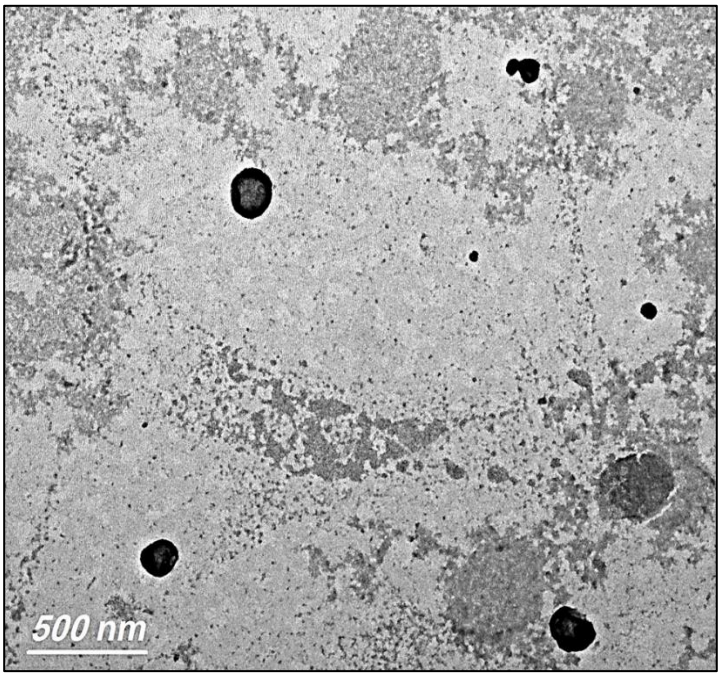

Fig. 2. Transmission electron microscopy of CLN.

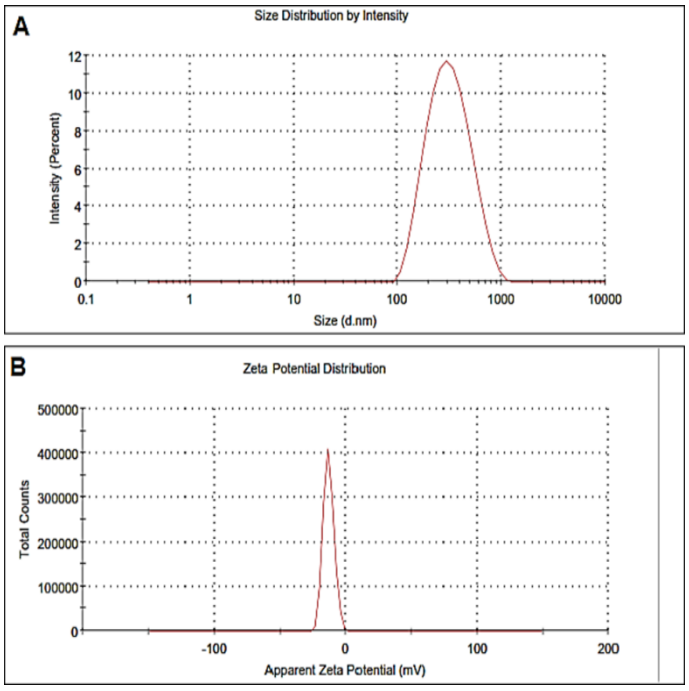

Fig 3. Particle size (A) and Zeta potential (B) of CLN.

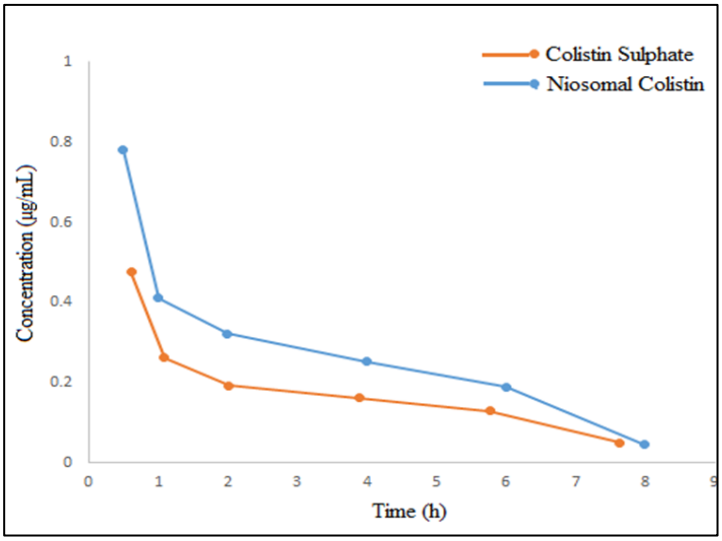

Fig. 4. Serum colistin concentration was measured after oral administration of 4 mg/kg of colistin and CLN, n=5.

Table 1. Serum colistin concentrations (µg/mL) following oral administration of colistin sulphate to broiler chickens.

	Single administration (4 mg/kg)	
Time (h)	**Colistin Sulphate (µg/mL)**	**Niosomal colistin (µg/mL)**
0	**N.A**	**N.A**
0.25		**0.81± 0.25**
0.5	**0.48 ± 1.25**	**0.78 ± 0.35****
1	**0.26 ± 0.86**	**0.412 ± 0.21***
2	**0.187 ± 0.58**	**0.322 ± 0.22***
4	**0.156 ± 0.46**	**0.253 ± 0.20***
6	**0.123 ± 0.18**	**0.189 ± 0.18**
8	**0.041 ± 0.13**	**0.046 ± 0.10**
9	**N.A**	**0.032 ± 0.12**
12	**N.A**	**N.A**
24	**N.A**	**N.A**

N.A: Not available**, star** indicates significances difference at the opposite time

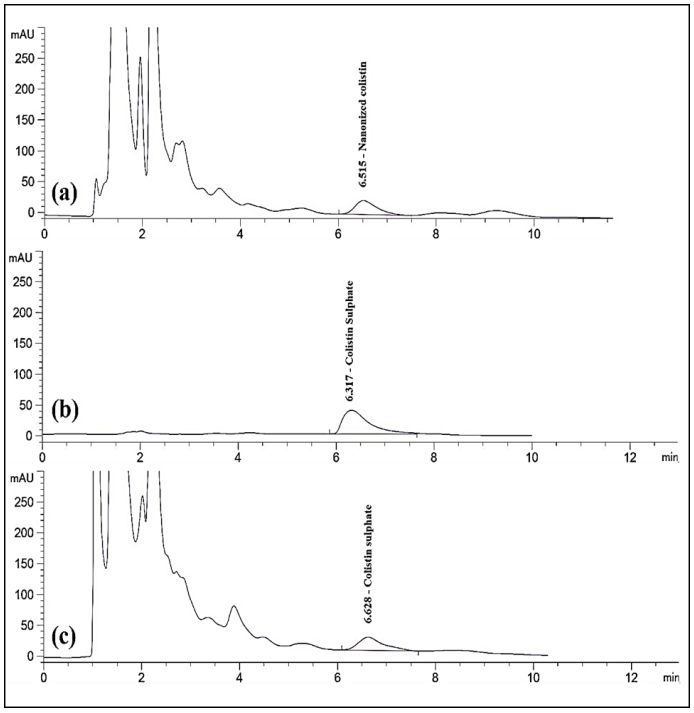

Fig. 5. HPLC chromatograms of Niosomal colistin **(a)** in serum sample of broiler chicken, standard colistin sulphate **(b),** colistin in a serum sample of broiler chicken **(c)**.

3.3. Estimation of Antimicrobial Efficacy of Colistin and CLN through MIC Estimation

MIC of colistin, CLN and cefotaxime against *E. coli* strain was 5µg/mL, 0.46µg/mL and 1.25µg/mL respectively compared with ciprofloxacin 22 µg/mL. MIC of CLN was lower by 12.5 ratios than that of free colistin demonstrating higher efficacy and that of free colistin. The statistical significance of $^{a}p<0.05$ was seen in comparison to the conventional colistin sulfate. Similarly, $^{b}p<0.05$ was found in relation to the niosomal colistin, additionally, $^{c}p<0.05$ was observed compared to cefotaxime.

3.4. Colistin and CLN *in vivo* Assay of Experimentally Infected Chicks

3.4.1. Clinical Signs, Mortality Rate and Postmortem Examination

Results of the experiment revealed no mortality in groups (G1, G3, G4 and G5) while, G2 and G6 recorded 13% and 6% mortality rates, respectively. All infected groups showed depression and off-food and yellow watery diarrhea which recovered with G4 and G6 more than other groups. Conversely, however, the necropsy of euthanized and dead chicks of each group found that (G1) appeared in normal condition, (G2) showed congestion of the GIT and turbidity of air sacs, caseous materials on heart, (G3) showed congestion of GIT, (G4) showed slight congestion of GIT only, (G5) showed congestion of kidney and deposition of ureates and (G6) showed congestion of kidney and deposition of ureates as showed in **Fig. (6).** Moreover, congested retained yolk sac in groups (G2, G3, G5 and G6) was observed until day 14 of age.

Fig. 6. Lesions of *E.coli* infections in different groups. As appeared congested GIT with bloody content and congested retained yolk sacs in G2, congested GIT and retained yolk sac in G3, slight congestion in GIT in G4 while (G5) and (G6) showed congestion of kidney and deposition of ureates (nephritis) and congested retained yolk sac.

3.4.2. Biochemical Assessments Pertaining to Renal Function

Regarding the biochemical assessments pertaining to kidney function, the blood urea nitrogen (BUN) concentrations and serum creatinine were determined after administration of conventional and niosomal colistin via oral and subcutaneously routes. Urea is impacted at a faster rate than creatinine; its concentration increased more in response to oral colistin than CLN; nevertheless, CLN significantly increased urea levels following injection in comparison to the control group. After being administered orally, CLN induced a substantial elevation in creatinine levels; conversely, subsequent injection led to a substantial reduction in creatinine levels **(Fig. 7).** Serum creatinine is a more accurate indicator of renal function than urea because it is more sensitive and its level reflects the true state of kidney function. Urea levels increase earlier in renal disease, whereas serum creatinine levels rise later**.**

Urea is rapidly affected before creatinine & increased more after oral colistin than CLN while after injection of CLN significantly increased the level than free colistin. At creatinine CLN increased the level significantly than oral free colistin while after injection; CLN significantly decreases the creatinine levels. The statistical significance of $^{a}p<0.05$ was seen in comparison to the negative control. Similarly, $^{b}p<0.05$ was found in relation to the Control positive infected birds, additionally, $^{c}p<0.05$ was observed compared to oral colistin. Columns bear no superscript or carry the same symbols indicates non-significant difference at p<0.05.

The albumin, globulin and total protein levels were determined in serum; following oral administration, there was a notable increase in the levels of albumin and total proteins, whereas there was no significant increase in the levels of globulin. Conversely, the total proteins, albumin and globulins levels decreased significantly, while the globulin levels did not significantly change **(Fig. 8).**

The statistical significance of $^{a}p<0.05$ was seen in comparison to the negative control. Similarly, $^{b}p<0.05$ was found in relation to the control positive infected birds, additionally, $^{c}p<0.05$ was observed compared to oral colistin. Columns bear no superscript or carry the same symbols indicates no discernible change at p<0.05.

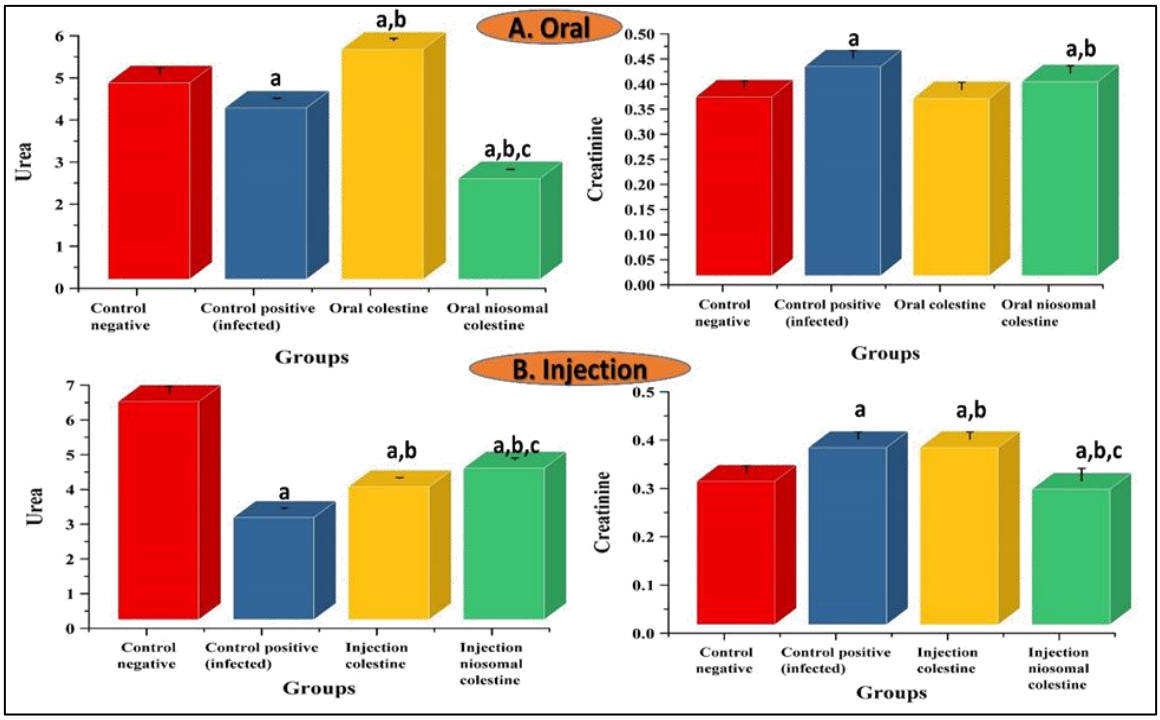

Fig. 7. Effects of free colistin and CLN on kidney function (Urea, creatinine) in comparison to normal and infected birds after last day of treatment. *E.coli* infected groups significantly affect the urea and creatinine levels in both oral and injected rats.

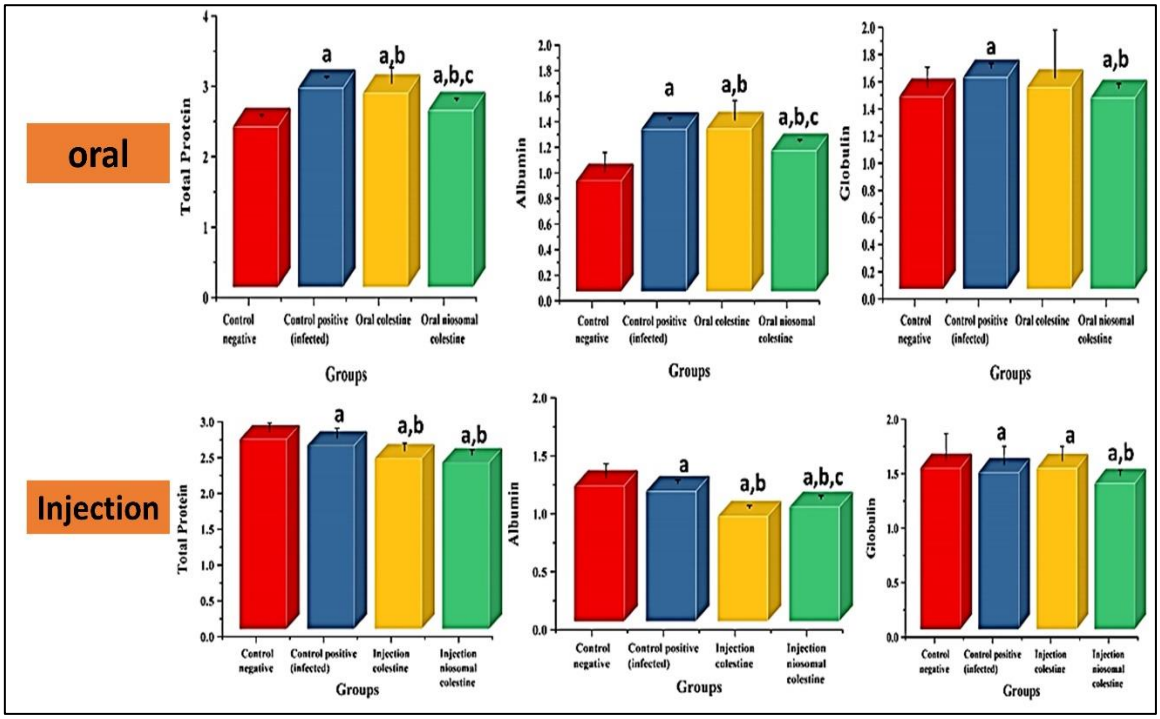

Fig. 8. Effects of colistin in conventional and niosomal form on serum total protein, albumin and globulin levels in comparison to normal and infected birds.

3.4.3. Histopathological Investigations

Histopathological investigations for the kidney, liver, heart and lung tissues are illustrated in **Figs. (9, 10, 11 and 12).**

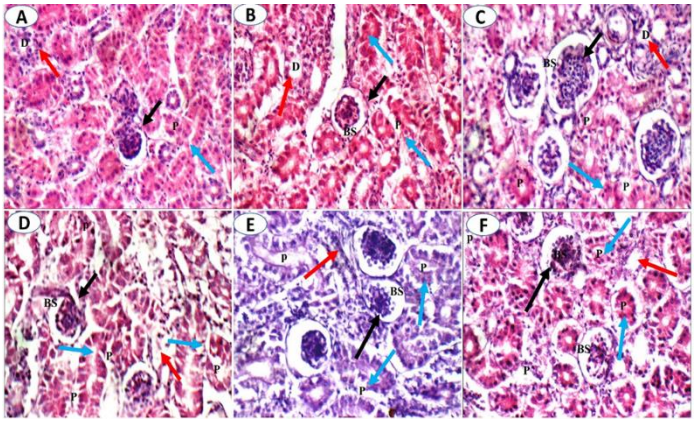

Fig. 9. Kidney tissue of varying treated groups, control negative (A), infected control positive (B), oral conventional colistin (C), oral niosomal colistin (D), parenteral conventional colistin (E) and parenteral niosomal colistin (F). All with high power with magnification of (H&E X400). All groups showed average glomeruli (Only Gr. B showed small-sized glomeruli) with average mesangial cells (black arrow) with average Bowman's spaces (BS), proximal tubules (P) and average distal tubules (red arrow). Average epithelial lining (A, F), markedly apoptotic epithelial lining (B, D), scattered apoptotic epithelial lining (C, E) were observed (blue arrow).

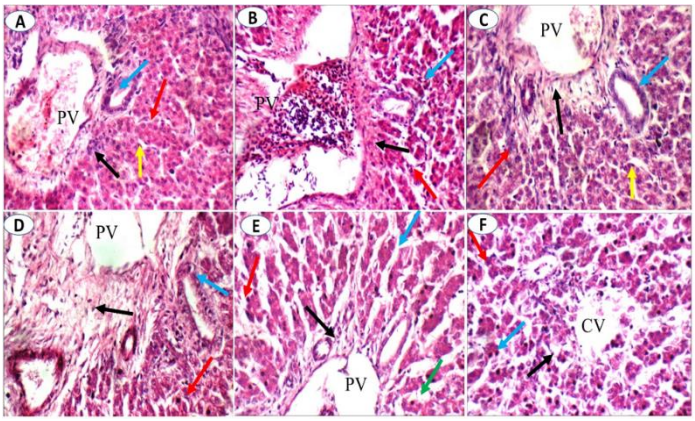

Fig. 10. Hepatic tissue of different treated groups; control negative (A), infected control positive (B), oral conventional colistin (C), oral niosomal colistin (D), parenteral conventional colistin (E), and parenteral niosomal colistin (F). All with high power with magnification of (H&E X400). A: Average portal tracts (black arrow) with average portal vein (PV), average bile ducts (blue arrow), average hepatocytes (red arrow), and average blood sinusoids (yellow arrow). B: Average portal tracts (black arrow) with mildly dilated congested portal vein (PV), scattered apoptotic hepatocytes (blue arrow), and average blood sinusoids (red arrow). C: Average portal tracts (black arrow) with mildly dilated portal vein (PV), average bile ducts (blue arrow), average hepatocytes (red arrow), and average blood sinusoids (yellow arrow). D: Mildly dilated portal vein (PV), average hepatocytes (blue arrow), and excess Kupffer cells (red arrow). E: Average portal tracts (black arrow) with average portal vein (PV), average hepatocytes (blue arrow), scattered Kupffer cells (green arrow), and mildly dilated blood sinusoids (red arrow). F: Average central vein (CV), normal hepatocytes (blue arrow), scattered Kupffer cells (blue arrow), and average blood sinusoids (red arrow).

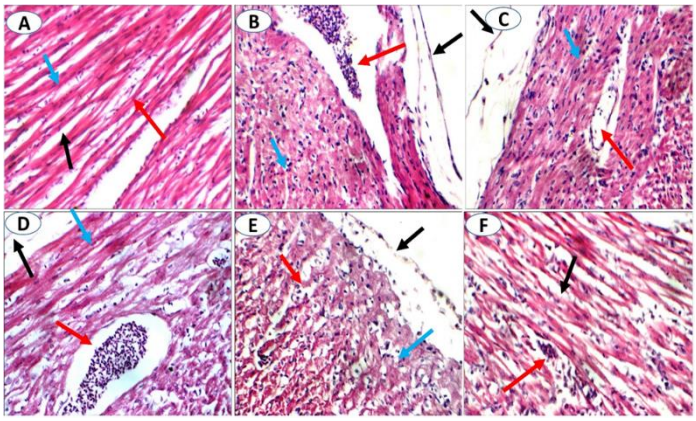

Fig. 11. Cardiac tissue of different treated groups; control negative (A), infected control positive (B), oral conventional colistin (C), oral niosomal colistin (D), parenteral conventional colistin (E), and parenteral niosomal colistin (F). All with high power with magnification of (H&E X400). A: Viable cardiac muscle fibers with distinct cell borders (black arrow) and central oval\elongated nuclei (blue arrow), and average interstitium (red arrow). B: Detached pericardium (black arrow), scattered apoptotic cardiac muscle fibers (blue arrow), and markedly dilated congested sub-pericardial blood vessels (red arrow). C: Average pericardium (black arrow), average cardiac muscle fibers (blue arrow), and average blood vessels (red arrow). D: Average pericardium (black arrow), normal cardiac muscle fibers (blue arrow), and mildly congested blood vessels (red arrow). E; Average pericardium (black arrow), scattered apoptotic cardiac muscle fibers (blue arrow), and average interstitium (red arrow). F: Average cardiac muscle fibers (black arrow), and mildly congested intervening blood capillaries (red arrow).

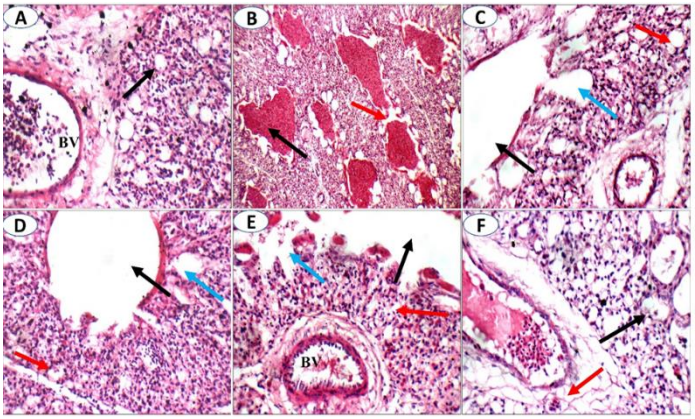

Fig. 12. Pulmonary tissue of different treated groups; control negative (A), infected control positive (B), oral conventional colistin (C), oral niosomal colistin (D), parenteral conventional colistin (E), and parenteral niosomal colistin (F). All with high power with magnification of (H&E X400). A: Average pneumocapillaries (black arrow), and average blood vessels (red arrow). B: Marked hemorrhages in Parabronchi (black arrow), and in pneumocapillaries (red arrow). C, D: Average Parabronchi (black arrow) with average infundibula (blue arrow), average pneumocapillaries (red arrow), and average blood vessels. E: Average Parabronchi (black arrow) with average infundibula (blue arrow), average pneumocapillaries (red arrow), and mildly congested blood vessels (BV). F: Average pneumocapillaries (black arrow) and mildly dilated congested blood vessels (BV) with mild peri-vascular edema (red arrow).

4. Discussion

The most dangerous side effect of colistin that calls for dosage modification is nephrotoxicity. Renal toxicity is caused by an elevation in the permeability of the epithelium lining the renal tubules, which results in acute tubular necrosis and cellular lysis **(Nation and Li, 2009)**. Previous research indicated that colistin was not well tolerated than other polymyxins, so its use was reduced to levels comparable to those of polymyxin B. Despite its notable bactericidal effectiveness, colistin and other polymyxins were gradually removed from clinical practice. On the other hand, the increased occurrence of bacterial MDR infections, has brought colistin back into the spotlight. At present time, colistin is regarded as an antibiotic of last resort in numerous medical domains where MDR is observed **(Falagas and Kasiakou, 2006; Tenover, 2006)**. A summary of the clinical and histopathological attributes of colistin-loading niosomes is presented in this study. Colistin-loaded noisome (CLN) was chosen for *in vitro* and *in vivo*

characterisation after a literature review **(Kazi et al., 2010; Chaw and Kim, 2013; Waddad et al., 2013).** These niosomes are being tested via nanotechnology in an effort to obfuscate or reduce the induced renal damage, which is a crucial factor in elucidating the potential mechanisms underlying colistin-induced nephrotoxicity. To achieve this, anionic polymers are utilized to reduce electrostatic interactions with kidney nerves or tissue.

After oral administration, the peaks of colistin were observed. The concentrations were 0.48µg/mL for free colistin and 0.78µg/mL for CLN at 0.5 h demonstrated that CLN of enhanced concentration which means significance increase of colistin serum level compared to free colistin. Higher (up to double fold) concentration of serum colistin was observed in CLN group than conventional colistin.

MIC of colistin sulphate, niosomal colistin and cefotaxime against *E. coli* strain was 5µg/mL, 0.46µg/mL and 1.25µg/mL respectively. While, it was 20µg/mL for ciprofloxacin which indicates the higher efficacy and bactericidal activity of niosomal prepared colistin than normal colistin form as a method for overcoming the colistin resistance and increasing efficacy. As MIC of niosomal colistin decreased by 12.5 double normal efficacy of colistin, more efficacy of NLC than colistin sulphate against resistant *E.coli* is predicted.

Chicks in the groups that received colistin via parenteral injection exhibited a substantially reduced concentration of serum total protein compared to the control group. There is lack of literature pertaining to the effects of high doses of colistin on serum total proteins, albumin and globulin in avian species **(Fitri et al., 2021; Gounden et al., 2023)**. Nevertheless, data does exist regarding colistin administration resulting in decreased serum concentrations of total proteins and albumin in rodents **(Yousef et al., 2012)**. In light of what we currently know, this is the first study looking into how colistin treatment affects total proteins, globulin, and serum albumin levels in broiler chickens.

Oral colistin increased urea levels more than niosomal colistin, whereas niosomal colistin substantially increased levels more than normal colistin after injection.Niosomal colistin significantly decreased creatinine levels compared to oral colistin, whereas oral colistin significantly increased creatinine levels. This finding suggests that colistin injections are safe for infected birds, as they reduce nephrotoxic effects. The clinical manifestations of colistin nephrotoxicity, unlike prior research, encompass a reduction in creatinine clearance and the possibility of proteinuria **(Florescu et al., 2012).**

Proximal tubule cells significantly absorb a significant amount of colistin. Colistin's polycationic nature makes it difficult to diffuse across the lipid bilayer at physiological pH values, suggesting that tubular reabsorption may be facilitated by transport systems. Research on colistin's transcellular transport mechanism is limited and recent, with its main nephrotoxicity mechanism primarily related to its poly cationic nature **(Li et al., 2003)**.

This investigation aimed to reduce nephrotoxic side effects associated with colistin, polymyxins, aminoglycosides, and other substances by coating colistin with anionic polymers. Colistin, a polymyxin, acts through cationic displacement and electrostatic interaction with negatively charged phospholipid head groups, leading to membrane instability, increased permeability, and cell death **(Shai, 1999; Yang et al., 2009).** The arrangement of renal brush-border membranes within the lipid bilayer varies significantly, despite the presence of anionic phospholipids in both prokaryotic and eukaryotic membranes. The outer leaflet of the bacterial membrane, where the negatively charged head group is exposed to the extracellular environment, is home to most anionic phospholipids. Negatively charged phospholipids in eukaryotic cells are divided into inner leaflets, resulting in less interaction between antibiotics and mammalian cell plasma membranes compared to bacterial membranes **(Matsuzaki, 1999)**. This study utilized liver, heart, and lung homogenates for comprehensive histopathological investigations due to their typically insensitive nature to colistin in vivo. The homogenization process may result in colistin having easier access to negatively charged phospholipids than it does in intact cells **(Gai et al., 2019).** Cholesterol, a eukaryotic bilayer component absent from bacterial membranes, can potentially decrease the antimicrobial activity of peptides by stabilizing the lipid bilayer or directly interacting with the peptide **(Matsuzaki, 1999).** This investigation suggests that niosomal preparations coated with anionic polymer are crucial for reducing interactions with the eukaryotic cell membrane, as direct colistin access could potentially disrupt membrane integrity **(Matsuzaki, 1999; Shai, 1999; Zasloff, 2002; Gai et al., 2019).**

The evaluation of histological abnormalities linked to colistin treatment is considered one of the most reliable methods for diagnosing colistin nephrotoxicity. This study's histopathological investigations confirm the safety of niosomal colistin on kidney and other body organs like liver, lung, and heart. After parenteral injection of colistin, kidney tissue showed normal glomeruli, mesangial cells, and Bowman's spaces, compared to conventional Colistin. Rats showed signs of tubular dilatation, vacuolation, and necrosis, without fibrous cicatrisation or inflammatory reactions **(Ghlissi et al., 2013).**

Niosomal colistin synthesis offers numerous benefits, including enhanced efficacy against resistant *E.coli*, increased serum level concentration and renal tissue protection from colistin's known nephrotoxic activity. Researchers are interested in antibiotic delivery systems using carriers like nano-polymerized particles, nano/micro emulsions, liposomes, and niosomes, as niosomes maintain intact molecules and protect them from environmental agents **(Kaur and Kumar, 2018; Akbarzadeh et al., 2021).** Niosomes are a novel, biodegradable and non-immunogenic drug delivery system that simultaneously delivers hydrophobic and hydrophilic drugs into the target tissue **(Amale et al., 2021; Naseroleslami et al., 2021; Targhi et al., 2021).** Niosomes, a type of lipid-based nanocarrier, are more efficient in storing non-ionic surfactants than phospholipid-containing liposomes. Niosomes offer superior features and benefits over liposomes, including enhanced chemical stability, enhanced biocompatibility, extended storage life, and improved handling **(Rajera et al., 2011; Bartelds et al., 2018).**

5. Conclusion

Colistin is expected to remain the last resort for severe Gram-negative infections due to rapid antibiotic development, multidrug-resistant infections, and potential nephrotoxicity in patients or animals. Research on preventing colistin-induced nephrotoxicity is crucial for optimizing colistin therapy efficacy. Mitigating nephrotoxic mechanisms by loading niosomes with anionic properties can reduce electrostatic interactions with renal tissue and prevent its nephrotoxicity. The current clinical requirement for colistin therapy in treating severe MDR infections is crucial. This study will greatly benefit clinicians by providing valuable insights for developing effective preventative and curative measures for colistin treatment.

6. Authors Contributions

All authors participated equally to the design of the research, methodology, and writing of the manuscript.

7. Conflict of Interest

The authors declare no conflict of interest.

8. References

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