Antibacterial Effect of Zinc Oxide and Copper Oxide Nanoparticles as Substitute of Antibiotics against Fowl Typhoid in Broilers

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ABSTRACT

Fowl typhoid has revolved into a pathogenic hazard to the poultry industry and extensive use of antibiotic is leading to the induction of antimicrobial resistance in microorganism. Main aim of this study was to find out substitute of antibiotic against Fowl Typhoid in broiler in terms of immunological, serum biochemistry and lipid profile parameters. Broiler chicks (Age = 1 d and n = 90) were kept under uniform management conditions. Chicks were divided randomly into six groups on day ten of their age with 15 replicates in each; control negative (CN), control positive (CP) and four treatments (T1, T2, T3 and T4). Whereas challenge infection of Salmonella gallinarum was given on nineteen days of age to all experimental birds except of those in CN group. On 22nd day, infected birds in T1 were given Florfenicol antibiotic. Whereas infected birds in groups; T2, T3 and T4 were given concentration of nanoparticles zinc oxide and copper oxide at different rate; 25+10, 37.5+15 and 50+20 mg/kg/d, respectively. Collected data were analyzed using complete randomized design through ANOVA technique. Mortality percent was found minimum 13.3% in birds under T3 group. Effect of all nanoparticle levels was not different (P>0.05) to that of antibiotic in terms of total serum protein and lipid profile; total glycerides, very low-density lipids, high density lipids and total cholesterol. It was concluded on the basis of findings of current study that nanoparticles zinc oxide and copper oxide mixture (37.5 + 15 mg/Kg/d) was found optimum alternate to Florfenicol antibiotic against Salmonella gallinarum infection in broiler birds. Hence, Zinc oxide and copper oxide nanoparticles could be an adequate alternative treatment replacing antibiotics against Fowl Typhoid in broilers.

INTRODUCTION

Poultry has become a dynamic livestock sub-sector that plays a crucial role in the economies of developing countries. It fulfills not only daily protein requirements of increasing human population in terms of high-quality food items like chicken meat and eggs but also triggers generation of employment sources. Globally, it has become a major source of food supplies around the world (Hussain et al., 2015). However, despite its rapid growth, the poultry industry faces numerous problems and bird mortality in particular is one of the main issues affecting the sustainability of poultry production worldwide. The mortality of birds might be due to spread of infectious diseases (Ahmed et al., 2022). These infectious diseases have become a huge risk to the poultry industry in terms of drug costs and consequently bird morbidity and mortality, resulting in high economic losses for any country (Abbas et al., 2015).

Fowl typhoid caused by a bacterium, Salmonella...
Salmonella gallinarum, is one of the pathogenic infectious diseases. The infectious diseases including fowl typhoid pose heavy economic losses to the poultry industry (Yasmin et al., 2019). Incubation period of this disease is about 4-6 days and occur in all types of birds with no exception of chicken of all ages. The birds suffering from fowl typhoid show depression, anorexia, dyspnea, weakness, droopy wings, ruffled feathers, huddling, adherence of droppings to the vent and diarrhea (Brennet et al., 2000). The birds shed the bacteria in droppings that cause the contamination of food and water (Nair et al., 2015). The disease can transmit both horizontally and vertically. The young chicks die within 5-10 days of hatching and mortality can reach up to 80% (Bhatti et al., 2013).

Antibiotics like Florfenicol, Enrofloxacin, Penicillin, Erythromycin, Oxytetracycline etc. are widely used against Salmonella species infection at poultry farms which leads to the induction of antimicrobial resistance (Oloso et al., 2019). The antimicrobial resistance (AMR) is the most important consequence of antimicrobial drugs used globally against Salmonella infection in animals. The antibiotics are used as metaphylactic and prophylactic treatment in the food producing animals. These agents are also used as growth promoters in broilers and other food animals. The AMR in food producing animals is of great concern (Threlfall, 2002). The irrational use of antimicrobial agents against non-typhoid Salmonella species is leading to the induction of antimicrobial resistance in microorganism. This evolutionary process makes the virulent strains able to survive in the unfavorable drug environment (Su et al., 2004). It has been foreseen that by 2050, antibiotic-resistant pathogens may cause about 10 million deaths worldwide (Castro-Vargas et al., 2020). The salmonella species can be transferred to the human being by handling or slaughtering the infected and morbid birds (Tizard, 2004; Mouttotou et al., 2017). The discovery of alternative, preventive and treatment methods could address the problem of antimicrobial resistance (AMR), as the WHO has issued a list of bacteria that have produced AMR (Oloso et al., 2019) and in the global plan of action 2015 instructions to overcome the problem and proposed to develop new drugs as antimicrobial solutions.

Nanotechnology could be a viable alternative solution for destroying fowl typhoid bacteria. The CuO and ZnO nanoparticles have sufficient bactericidal activity against a variety of gram-positive and gram-negative bacteria (Zarrindokht and Pegah, 2011; Das et al., 2013; Khashan et al., 2016) and these metal oxides ions like Zn$^{2+}$ and Cu$^{2+}$ which react with the negatively charged bacterial cells. Reactive oxygen species are produced by the nanoparticles, which bind to the bacterial cell wall, enter the cell and consequently destroy the bacterial cell (Ahmed et al., 2022). The nanoparticles (NPs) can also cause bacterial cell death by destroying the vital enzymes in the bacterial cells (Dadi et al., 2019) and after entering into the bacterial cell, ZnO NPs interact with the sulphur and phosphorus containing compounds like DNA of the bacterial cell leading to bacterial cell death (Raguvaran et al., 2015).

Keeping in view the importance of ZnO and CuO nanoparticles as an adequate alternative technique, the present study was designed with main aim to find out the optimum alternative treatment solutions replacing antibiotic. The specific objective of present study was to examine the antibacterial effect of different levels of ZnO and CuO nanoparticles in comparison with Florfenicol against Salmonella gallinarum induced infection in broilers by immunological, serum biochemistry and lipid profile parameters.

**MATERIALS AND METHODS**

**Experimental site**

Experiment was conducted in Pathology Department, Faculty of Veterinary Science, University of Agriculture Faisalabad, Punjab, Pakistan. The geographical coordinates of research site are; Latitude: 31° 25' 46.8048". Longitude: 73° 4' 14.3112". Latitude: N 31° 25.7801°. Longitude: E 73° 4.2385°. Climate of the experimental site was cold with foggy nights. Ambient temperature ranged from 10 to 20°C whereas average relative humidity was 66%. The nanoparticles were synthesized in the Department of Physics. Experimental trial was conducted for a period of 30 days w.e.f. 24th December 2020 to 22nd January 2021 in poultry shed of Parasitology Department, whereas different laboratories in Faculty of Veterinary Science; Disease Diagnostic Laboratory, Physiology Laboratory and Molecular Pathology Laboratory, were used for lab analysis in this study.

**Treatment groups**

One day-old broiler chicks (n= 90) were taken from a local commercial hatchery. All the experimental birds were homogenous with no visible variation regarding age, weight and size. According to ethical standards, experimental birds were given freedom from hunger and thirst as well as provided an environment in which birds expressed normal behavior. Feeding was provided uniformly to all animals as per their requirements under uniform housing and management conditions. On day 10, the birds were randomly divided into six groups; control negative, control positive, treatment 1, treatment 2, treatment 3 and treatment 4 (CN, CP, T$_1$, T$_2$, T$_3$, and T$_4$).
Each treatment group comprised 15 birds and kept under different individual compartments. The vaccination of the birds was administered against ND and IB on 3rd and 14th days of age.

**Induction of infection**

On day 19, the challenge of *Salmonella gallinarum* was given to the birds of all groups except control negative (CN) group at dose 10⁸ CFU/ml via crop route method. The group was T₁ was given treatment florfenicol and groups T₂, T₃, and T₄ were given treatments of ZnO and CuO nanoparticles at different dose level 25+10, 37.5+15 and 50+20 mg/kg/d respectively as shown in the experimental layout (Table I). The treatment was given to the birds after the appearance of clinical signs (3 days post infection).

**Parameters studied and data collection**

Mortality ratio of the birds were noted between 19th and 30th day, whereas three birds from each group were slaughtered for sample collection on 26th and 30th day of trial. In order to separate the serum, the blood samples were collected in 5 ml syringes and kept in a hot air oven (37° C) for 30 min. In a 1.5 ml Eppendorf tube, the serum was collected and kept at -20° C for 15 days. The total serum proteins were determined by using Bioclin Kit, Brazil, LOT-1038 and serum albumin were determined by using Bioclin Kit, Brazil, LOT-0127. The serum globulin was determined by subtracting serum albumin from total serum proteins using following equation.

\[
\text{Serum globulin} = \text{Total serum proteins} - \text{Serum albumin}
\]

The lipid profile (triglycerides, high density lipids, low density lipids, very low-density lipids and total cholesterol) was determined by using commercially available kits. The triglyceride level was determined by using LabKit, Spain, LOT: LIQ-418-A, HDL-C was determined by using Human Diagnostic Kit, Germany, LOT: 0072 and total cholesterol was determined by using Human Diagnostic Kit, Germany, LOT: 0166. The VLDL were determined by dividing the triglyceride value by 5 by using following equation.

\[
\text{VSL} = \frac{\text{Triglycerides}}{5}
\]

Low density lipid cholesterol was also calculated by following equation.

\[
\text{LDL} = \text{Total Cholesterol} - \left( \frac{\text{Triglycerides}}{5} + \text{HDL} \right)
\]

**Antibody titer against sheep RBCs**

The antibody response against sheep RBCs was determined as described previously (Delhanty and Solomon, 1966). The 3% washed sheep RBCs were injected to the birds on 14th and 21st day of experiment. The antibody titer against sheep RBCs was determined from the serum collected from birds in all treatment groups on 21st and 28th day of trial. The sheep blood was collected in the EDTA vacutainer (Lab Vac. LOT: 07072014) from jugular vein of a sheep maintained at UAF Small Ruminant Farm using a sterile syring. After washing the sheep RBCs, a 3% (V/V) suspension of sheep RBCs was used.

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Table I. List of experimental operations at different age days of experimental broilers under treatment groups.

<table>
<thead>
<tr>
<th>Age (day)</th>
<th>Control Negative</th>
<th>Control Positive</th>
<th>Treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Division of birds into 6 Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1st Injection of Sheep RBCs to birds in all groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Nil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Collection of Serum from Sheep RBCs injected Birds and 2nd Injection of Sheep RBCs to birds in all groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Sampling-I (7 days after infection of <em>S. gallinarum</em>): Humane Slaughtering of Birds, Collection of Blood, Serum Separation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Sampling-II (11 days after infection of <em>S. gallinarum</em>): Humane slaughtering of birds, collection of blood, serum separation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each treatment group comprised 15 birds and kept under different individual compartments. The vaccination of the birds was administered against ND and IB on 3rd and 14th days of age.
prepared in the normal saline. On day 14th and 21st, 1 ml of 3% sheep RBCs were injected aseptically in wing vein of birds of all groups and the serum was separated from the blood taken from the injected birds after 7-days and 14-days post injection. The antibody titer against sheep RBCs was determined by testing the collected serum samples using the titration method following the inactivation of serum in hot air oven. A volume of 50 µL of phosphate buffer saline was added in well of the row of microtitration plate for each sample. A volume of 50 µL of inactivated serum sample was added in the 1st well of the microtitration plate and incubated for 30 min at 37°C. After 30 min incubation, two-fold serial dilution was done for each sample. A volume of 50 µL of 3% sheep RBCs were added into each well and incubated at 37°C for 30 min and the titers were noted down. The treatment of collected serum with 2-merceptoethanol was done to determine the IgM and the level of IgG was determined from total antibody response minus IgM.

**Statistical analysis**

The collected data were analyzed under complete randomized design through ANOVA technique, whereas group mean comparison was made through Tukey’s test (Steel et al., 1997) using SAS® University Edition online software SAS 15.1.

**RESULTS**

Effect of Florfenicol antibiotic and varying treatment levels of nanoparticles was determined in the experimental birds with induced infection of *Salmonella gallinarum* in terms of following studied parameters serum biochemistry, immunoglobulins and mortality percentage in Tables II, III and Figure 1, respectively.

**Mortality rate**

Effect of different levels of nanoparticles with comparison of Florfenicol antibiotic in terms of mortality rate is mentioned in Figure 1. Mortality was found Nil in control negative group, whereas it was at its highest i.e., 60% in control positive group. However, 20% mortality was observed in birds treated with Florfenicol, whereas 20%, 13% and 20% mortality was found in birds treated with nanoparticle treatment groups; T3, T4 and T5 respectively. The lowest mortality was found in treatment T1: ZnO 37.5 + CuO 15 mg/kg/d.

**Table II. Antibacterial effect of varying levels of mixed zinc oxide and copper oxide nanoparticles and Florfenicol on Salmonella gallinarum induced infection in broiler in terms of serum biochemistry and lipid profile at 7th day and 11th day post infection.**

<table>
<thead>
<tr>
<th>Serum biochemical parameters</th>
<th>Control negative</th>
<th>Control positive</th>
<th>T1: Florfenicol</th>
<th>Nanoparticle levels of ZnO and CuO (mg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum proteins (mg/dl)</td>
<td>S1 4.42 ± 1.35a</td>
<td>1.45 ± 0.39a</td>
<td>3.43 ± 0.65a</td>
<td>3.42 ± 0.59a 3.48 ± 0.46a 3.28 ± 0.31a</td>
</tr>
<tr>
<td>Serum albumin (mg/dl)</td>
<td>S1 3.50 ± 0.73a</td>
<td>1.11 ± 0.11a</td>
<td>3.22 ± 0.72a</td>
<td>1.79 ± 0.32a 1.76 ± 0.34a 1.99 ± 0.11b</td>
</tr>
<tr>
<td>Serum globulin (mg/dl)</td>
<td>S1 0.92 ± 0.70a</td>
<td>0.34 ± 0.37a</td>
<td>1.12 ± 0.21a</td>
<td>1.62 ± 0.37a 1.71 ± 0.17a 1.29 ± 0.29a</td>
</tr>
<tr>
<td>Total glycerides (mg/dl)</td>
<td>S1 482.05 ± 38.71a</td>
<td>251 ± 38.71a</td>
<td>446.15 ± 15.38a</td>
<td>533.33 ± 47.0b 548.71 ± 38.71a 569.23 ± 55.47a</td>
</tr>
<tr>
<td>Very low density lipids (mg/dl)</td>
<td>S1 96.41 ± 7.74a</td>
<td>50.25 ± 7.74a</td>
<td>89.23 ± 3.07b</td>
<td>106.6 ± 9.4b 109.74 ± 7.74a 113.84 ± 11.09a</td>
</tr>
<tr>
<td>High density lipids (mg/dl)</td>
<td>S1 93.07 ± 27.13a</td>
<td>31.92 ± 10.25a</td>
<td>65.89 ± 8.48a</td>
<td>94.42 ± 16.47a 83.55 ± 9.33a 101.22 ± 27.13a</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>S1 277.17 ± 26.76a</td>
<td>135.86 ± 44.89a</td>
<td>231.88 ± 22.13a</td>
<td>289.85 ± 38.46a 294.38 ± 46.61a 304.34 ± 52.94a</td>
</tr>
<tr>
<td>Low density lipid (mg/dl)</td>
<td>S1 87.69 ± 44.7a</td>
<td>53.68 ± 30.34a</td>
<td>76.75 ± 22.61a</td>
<td>88.75 ± 30.27a 101.08 ± 56.84a 89.27 ± 7.71a</td>
</tr>
</tbody>
</table>

Note: Mean Values in rows with various superscripts are different (P<0.05), S1 and S2 indicates Sampling 1 and Sampling 2.
Table III. Antibacterial effect of varying levels of mixed zinc oxide and copper oxide nanoparticles and Florfenicol on *Salmonella gallinarum* induced infection in broiler in terms of Ig, IgG and IgM.

<table>
<thead>
<tr>
<th>Response After 1st Injection</th>
<th>Control Negative</th>
<th>Control Positive</th>
<th>T1: Florfenicol</th>
<th>T2: (25 + 10)</th>
<th>T3: (37.5 + 15)</th>
<th>T4: (50 + 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ig</td>
<td>5.5 ± 0.71\a\b</td>
<td>4.5 ± 0.71\a\b</td>
<td>5.5 ± 0.71\a\b</td>
<td>6.5 ± 0.71\a</td>
<td>6.5 ± 0.71\a</td>
<td>6.5 ± 0.71\a</td>
</tr>
<tr>
<td>IgG</td>
<td>4.5 ± 0.71\a\b</td>
<td>3.0 ± 1.41\a\b</td>
<td>4.5 ± 0.71\a\b</td>
<td>5.0 ± 0\a\b</td>
<td>5.5 ± 0.71\a</td>
<td>5.5 ± 0.71\a</td>
</tr>
<tr>
<td>IgM</td>
<td>1 ± 0\a\b</td>
<td>1.5 ± 0.71\a\b</td>
<td>1.0 ± 0\a\b</td>
<td>1.5 ± 0.7\a\b</td>
<td>1.0 ± 0\a\b</td>
<td>1.0 ± 0\a\b</td>
</tr>
<tr>
<td>Response After 2nd Injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Ig</td>
<td>6.0 ± 0\a\b</td>
<td>4.5 ± 0.71\a\b</td>
<td>6.0 ± 1.41\a\b</td>
<td>6.5 ± 0.71\a\b</td>
<td>5.0 ± 1.41\a\b</td>
<td>5.5 ± 0.71\a\b</td>
</tr>
<tr>
<td>IgG</td>
<td>4.5 ± 0.71\a\b</td>
<td>3.5 ± 0.71\a\b</td>
<td>4.5 ± 0.71\a\b</td>
<td>5.5 ± 0.71\a\b</td>
<td>4.5 ± 2.12\a\b</td>
<td>4.5 ± 0.71\a\b</td>
</tr>
<tr>
<td>IgM</td>
<td>1.5 ± 0.71\a\b</td>
<td>1.0 ± 0\a\b</td>
<td>1.5 ± 0.71\a\b</td>
<td>1.0 ± 0\a\b</td>
<td>0.5 ± 0.71\a\b</td>
<td>1.0 ± 0\a\b</td>
</tr>
</tbody>
</table>

\*abc Mean Values in rows with various superscripts are different (P<0.05).

Serum albumin

At 7\textsuperscript{th} day post infection, serum albumin was decreased (p<0.05) in control positive group as compared to control negative group. The serum albumin was higher through antibiotic (Florfenicol) therapy. The serum albumin was higher (p<0.05) in T\textsubscript{1} as compared to that of control positive group. The serum albumin of treatment groups T\textsubscript{2}, T\textsubscript{3} and T\textsubscript{4} was found not different (p>0.05) to that of control positive group. At 11\textsuperscript{th} day post infection, the serum albumin was decreased (p<0.05) in control positive group as compared to control negative group. The serum albumin in T\textsubscript{1} (Florfenicol) and T\textsubscript{4} was improved numerically. The serum albumin in treatment groups T\textsubscript{2}, T\textsubscript{3} and T\textsubscript{4} was found comparable (p>0.05) to that of T\textsubscript{1}.

Serum globulins

At 7\textsuperscript{th} day post infection, the serum globulins of groups T\textsubscript{2} and T\textsubscript{4} were found higher (p<0.05) as compared to that of control positive group while the serum globulins of T\textsubscript{1} and T\textsubscript{3} was found numerically higher (p<0.05) to that of control positive group. The serum globulin concentration in nanoparticle treated groups (T\textsubscript{2}, T\textsubscript{3} and T\textsubscript{4}) was found comparable (p>0.05) to that of treatment group T\textsubscript{1} (Florfenicol). However, at 11\textsuperscript{th} day post infection, the serum globulins were found not different (p>0.05) in all groups (CN, CP, T\textsubscript{1}, T\textsubscript{2}, T\textsubscript{3} and T\textsubscript{4}).

Total glycerides

At 7\textsuperscript{th} day post infection, the total glycerides were decreased (p<0.05) in control positive group as compared to control negative group. The total glycerides were recovered through antibiotic (Florfenicol) therapy. The total glycerides were higher (p<0.05) in T\textsubscript{1} as compared to that of in control positive group. The total glycerides in the groups T\textsubscript{2}, T\textsubscript{3} and T\textsubscript{4} were found lower (p<0.05) than that of control positive group. The total glycerides of treatment groups T\textsubscript{2} and T\textsubscript{3} were found comparable to

Fig. 1. Antibacterial effect of nanoparticles and antibiotic on mortality percentage in broilers.

**Total serum proteins**

At 7\textsuperscript{th} day post infection, the total serum protein was decreased (p<0.05) in control positive group as compared to control negative group. The total serum protein was higher in the group treated with antibiotic (Florfenicol) therapy. The total serum proteins were higher (p<0.05) in T\textsubscript{1} as compared to that of control positive group. The serum albumin was decreased (p<0.05) in control negative group. The serum albumin was lower (p<0.05) than that of control positive group whereas total serum proteins of only treatment group T\textsubscript{1} were found different as compared to that of control positive group. However, the total serum proteins of nanoparticles treated groups (T\textsubscript{2}, T\textsubscript{3}, T\textsubscript{4}) were found comparable (p>0.05) to that of T\textsubscript{1} (Florfenicol) group.
that of T4, (Florfenicol). At 11th day post infection, the total glycerides were decreased (p<0.05) in control positive group as compared to control negative group. The total glycerides were recovered through antibiotic (Florfenicol) therapy. The total glycerides were higher (p<0.05) in T3 as compared to that of in control positive group. The total glycerides in the groups T1, T2, and T4 were found higher (p<0.05) to that of control positive group. The total glycerides of treatment groups T1, T2, and T4 were found comparable (p>0.05) to that of T1 (Florfenicol).

**Very low-density lipids**

At 7th day post infection, very low-density lipids were decreased (p<0.05) in control positive group as compared to control negative group. Very low-density lipids were improved through antibiotic (Florfenicol) therapy. Very low-density lipids were higher (p<0.05) in T1 as compared to that of in control positive group. Very low-density lipids of nanoparticles treated (T1, T2 and T3) groups were found higher (p>0.05) as compared to that of control positive group. Very low-density lipids of nanoparticles treated (T1 and T2) groups were found comparable (p>0.05) to that of group T1 (Florfenicol) whereas very low-density lipids of treatment group T3 was found higher (p>0.05) as compared to that of T1 (Florfenicol). At 11th day post infection, very low-density lipids were decreased (p<0.05) in control positive group as compared to control negative group. Very low-density lipids were recovered through antibiotic (Florfenicol) therapy. Very low-density lipids were higher (p<0.05) in T1 as compared to that of in control positive group. Very low-density lipids of nanoparticles treated (T1, T2 and T3) groups were found different (p<0.05) as compared to that of control positive group. Very low-density lipids of nanoparticles treated (T2, T3, and T4) groups were found comparable (p<0.05) to that of group T1 (Florfenicol).

**High density lipids**

At 7th day post infection, high density lipids were decreased (p<0.05) in control positive group as compared to control negative group. High density lipids were improved numerically in the group T1. High density lipids in treatment groups T2, T3, and T4 were found different (p<0.05) to that of control positive and comparable to that of T1 (Florfenicol). At 11th day post infection, high density lipids were decreased (p<0.05) in control positive group as compared to control negative group. High density lipids were improved numerically in the group T1. High density lipids in treatment groups T2, T3, and T4 were found higher (p<0.05) to that of control positive and comparable (p>0.05) to that of T1 (Florfenicol).

**Total cholesterol**

At 7th day post infection, total cholesterol was decreased (p<0.05) in control positive group as compared to control negative group. High density lipids were improved numerically in the group T1. The total cholesterol of treatment groups T2, T3, and T4 was found different (p<0.05) as compared to that of control positive group and comparable (p>0.05) to that of T1 (Florfenicol) treatment group. At 11th day post infection, total cholesterol was decreased (p<0.05) in control positive group as compared to control negative group. Total cholesterol was higher (p<0.05) in the group T1 as a result of antibiotic (Florfenicol) therapy. The total cholesterol of treatment groups T2, T3, and T4 was found higher (p<0.05) as compared to that of control positive group and comparable (p>0.05) to that of T1 (Florfenicol) treatment group.

**Low density lipids**

At 7th day post infection, low density lipids were decreased (p<0.05) in control positive group as compared to control negative group. Low density lipids were improved numerically in the group T1. Low density lipids of treatment groups T2, T3, and T4 was found higher (p<0.05) as compared to that of control positive group and comparable (p>0.05) to that of T1 (Florfenicol) treatment group. At 11th day post infection, low density lipids of all groups were found not different (p>0.05).

**Antibody titer against sheep RBCs**

The log antibody titer (Ig, IgG and IgM) of all treatment groups (T1, T2, T3, and T4) including control negative and control positive against sheep RBCs after 1st and 2nd injection was found not different (p>0.05) as mentioned (Table III). However, IgG in birds treated with T1 was found higher (p<0.05) than that of control positive treatment in case of 2nd injection of washed Sheep RBCs. While in case of treatment groups T2, T3 and T4, the IgG level was found comparable (p>0.05) to that of control positive group. The IgG level in nanoparticles treated groups (T2, T3, and T4) was comparable to that of treatment group T1 (Florfenicol).

**DISCUSSION**

In continuation of previous efforts, findings of this study also substantially endorsed nanotechnology as a reasonable substitute of antibiotic treatment against fowl typhoid. Preliminary findings like clinical signs appeared in response to induced infection of Salmonella gallinarum like fatigue, loss of appetite, ruffled feathers, sunken eyes, yellow diarrhea and significant mortality were in line to with the previous studies (Shivaprasad, 2000; Shah et
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al., 2013; Chiroma et al., 2017; Birhanu et al., 2020). In addition to above, gross pathological signs like bronze colored liver, splenomegaly and necrotic foci on visceral organs; liver, spleen and heart were also validated by Kumari et al. (2013). Followed by signs, significant decline in level of serum biochemistry parameters like total serum proteins, serum albumin and serum globulins was reported by previous workers (Kokosharov, 2006; Shah et al., 2013; Fotouh et al., 2014; Biazus et al., 2017). This decile might be due to damaged liver resulting in decreased synthesis of plasma proteins and serum albumin (Biazus et al., 2017), whereas damaged kidney led to increased loss of proteins and decreased appetite (Coles, 1980). In fact catalase enzyme might be produced by Salmonella gallinarum which could trigger proteolysis and consequently it might reduce protein concentration in the blood (Kokosharov, 2000).

Nanoparticles might be substantiated substitute of Florfenicol antibiotic therapy due to its adequate efficacy against induced infection of Salmonella gallinarum as evident by findings of this study. Total serum proteins and globulin level was preliminary decreased in response to infection and then reinstated might be attributed to a factor of substantial response of nanoparticles under T2 and T3 treatments at 1st sampling. This might be due to bactericidal activity of nanoparticle by degenerating the bacterial cells (Dadi et al., 2019; Ahmed et al., 2022) which could prevent liver damage in case of treated birds.

Comparable efficacy of nanoparticles with that of antibiotic in terms of lipid profile was also noticed. Following pattern of serum proteins and globulin, lipid profile parameters; total cholesterol, triglycerides, high density lipids, low density lipids and very low density lipids were decreased and then reinstated. This might be attributed due to a factor that liver tissue exposed to nanoparticle treatment could significantly increase lipid peroxides formation (Syama et al., 2013). Furthermore, Ahmadi et al. (2013) also strengthened findings of this study that lipid profile in terms of cholesterol, HDL and LDL were increased by nanoparticle treatment in birds.

The log antibody titer against sheep RBCs was found not different (P>0.05) in our study in all treatment groups at the time of both samplings (7th and 11th days post infection). These findings were in line to a recent study of Ahmed et al. (2022). However, Bami et al. (2018) reported differently in contrast to findings of this study that there was an increase in the log antibody titer against sheep RBCs in birds supplemented with ZnO NPs. The reason for this divergence might be due to different methodology adopted in that study in which birds were not induced infection of Salmonella gallinarum. Further research is recommended to authenticate the efficacy of nanoparticle treatment in birds infected with Salmonella gallinarum.

It was inferred based on fact of comparable efficacy of nanoparticles with that of Florfenicol against fowl typhoid, any dose level of zinc oxide and copper oxide 25+10, 37.5+15 and 50+20 mg/kg/d could be used as substitute of antibiotic treatment. However, a combination of zinc oxide and copper oxide with concentration 37.5 + 15 mg/kg/d was found optimum level of nanoparticles based on findings regarding minimum mortality of birds infected with Salmonella gallinarum in this treatment group. In general, nanoparticles could be a potential technique to be industrialized and applied in poultry industry to save birds from the danger of fowl typhoid with no antibiotic. Replacing antibiotic with more friendly and safer treatment with nanoparticles could ensure food safety for human consumption.

CONCLUSION

It was concluded on the basis of findings of current study that nanoparticles zinc oxide and copper oxide mixture (37.5 + 15 mg/Kg/d) was found optimum alternate to Florfenicol antibiotic against Salmonella gallinarum infection in broiler birds. Hence, Zinc oxide and copper oxide nanoparticles could be an adequate alternative treatment replacing antibiotics against fowl typhoid in broilers. Further research is required to authenticate findings of present study. It is also recommended that replacing antibiotic with administration of nanoparticles through water should be studied which would be more easy for farmers for implementation.

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IRB approval

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**Ethical statement**

During the whole period research trail, the bird were provided freedom from hunger, thirst and pain. A suitable environment was provided to express the natural behavior.

**Statement of conflict of interests**

The authors have declared no conflict of interest.

**REFERENCES**


