Effect of Chitosan Nanoparticles Loaded Oxytetracycline Hydrochloride on Health Status of Common Carp *(Cyprinus carpio L.)* Infected with Columnaris Disease

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Abstract. This study was aimed to evaluate the effect of chitosan nanoparticles loaded oxytetracycline hydrochloride on health status, and survival rate of common carp infected with Columnaris disease. A total 160 fish of *Cyprinus carpio* L. weighed between 48-50g were randomly distributed into eight treatments, and 48 fish were used to determine LD50. (Two replications for each treatment). The bacterial concentration of *F. columnara* used for infection test after a serial dilution was 0.2ml (1.8 × 106 CFU/ml), then removal a few of scales near the caudal fin and injection subcutaneously. The trial injected fish treated by ordinary oxytetracycline hydrochloride as T0 in dose 20 mg /L and T1, T2, T3, T4 and T5 treated by chitosan nanoparticles loaded oxytetracycline hydrochloride in dose 20, 15, 10, 5, 2.5 mg/L respectively. The control (+) infected without gave treatment, whilst, control- was no infected. All the treatments gave the bath treatment 1-hour per-day for 7 days, clinical signs post treatment were evaluated also, RBC counts, WBC counts, PCV, Hb, Total proteins, Albumin, Globulin, A/G ratio and survival rate were measured. One week after the end of the treatment period, an improvement in health and feed consumption was observed in T4 and T3. The results revealed significant increases (P < 0.05) in PCV, Hb, RBCs count and WBC count of *C. carpio* post-treatment in treated groups T4 (26.46%, 8.95 g/dl and 2.27 cell×10⁶/mm³ and 22.10 cell×10³/mm³ respectively) compared with control (+) (15.95%, 5.94 g/dl, 1.55 cell×10⁶/mm³ and 19.13 cell×10³/mm³ respectively). In addition, Total proteins, Albumin, Globulin showed significant increase (P < 0.05) in T2, T3, T4, T5 comparison with the control+ group and highest values were recorded in T4. While, The result of A/G ratio revealed a significantly decreased (P > 0.05) in all treatments in compared with Control(-) and no significant difference (P > 0.05) compared with Control(+). The best A/G ratio recorded in T4 and T5. In addition, survival rate 90% recorded as the highest value in T4. This current administration technique of oxytetracycline, proved to become much more efficient with respect to conventional exposure and has the potential to minimize the usage of antibiotics in fish farms and regarding environmental effects.

Keywords: chitosan, nanoparticles loaded, oxytetracycline, health status, common carp, columnaris

1. Introduction

Common carp (*Cyprinus Carpio L.*) is known to be the most important aquaculture fish in many Asian and European countries (Mohammad, 2015). It is introduced into Iraq as a breeding fish and transmitted among all Iraqi water bodies since it is living in different environmental conditions, has the fastest growth at water temperatures around 23-30 C, survives in cold winter, tolerates salinity of...
12 g / L (Al-Hamed, 1971; Al-Rudainy et al., 2008). The production of fish farming causing massive economic losses each year worldwide is regarded as being considerably limited by the outbreak of disease (Austin, 2007). The infections of bacterial are a major impendence to economic viability for any aquaculture farming (Yousefian et al., 2009). Fish are sensitive to a wide range of infectious bacteria causing a high mortality rate in fish culture (El-Sayyad et al., 2010). Columnaris disease is one of the most common infectious bacterial diseases of freshwater fishes infecting both cultured and wild fish species. It has a huge economic effect on fish farming due to the increased and rapid mortality rates of it creates. (Marshet, 2018). Flavobacterium columnare is a widely available bacterium in aquatic ponds as well as a causative agent of Columnarisis disease in fine fish. Flavobacterium columnare has also been recognised as a global bacterium for fish species (Figueiredo et al., 2005).

The administration of antibiotics, for many years, was the generality common method for handling the occurrence of bacterial infection in the aquatic ecosystem. However, dangerous problems still face the aquaculture because of adverse results of those drugs like accumulate in the tissue of creatures, immunosuppressant, development of bacteria to resistant antibiotic and ruin of environmental microbial flora (Baulny et al., 1996). Due to the increase in the outbreak of bacterial diseases in the aquaculture industry and the development of bacterial resistance, new antibacterial agents are required (Gong et al., 2007; Soltani et al., 2009). The nanoparticles have been used to deliver the drugs into the cells with negligible side effects (Scott, 2005). Chitosan nanoparticles and PLGA nanoparticles are being tested as nanoparticles for drug delivery in fish medicine. (Wang, 2011). Chitosan is a mucopoly saccharide that is closely related to cellulose. It is a derivative of chitin and is formed by chitin deacetylation. (Soutter, 2013; Crini, 2019).

Chitosan nanoparticles were used to boost their transfer efficiency in cells as drug carriers and gene carriers, as recorded in many researchers (Chopra et al., 2014; Csaba and Alonso, 2014). In the Fish culturists notified that the oxytetracycline-hydrochloric acid bath handlings were, efficient in decreasing mortality in freshwater fish diseased with F. columnare (Stehly 2002). Oxytetracycline-hydrochloric successfully minimize the mortality in walleyes (10 mg/L only) and channel catfish infected with Columnaris (Rach et al., 2008). The synthesis of antibiotic conjugates with biopolymers is a relatively new trend of research that generally has the purpose of prolongation the antibiotic action and decreasing its toxicity (Ueda et al., 1989).

In this current study explained the effect of chitosan nanoparticles loaded with oxytetracycline on some blood parameters and health status of carp fish with columnaris disease in comparison with conventional oxytetracycline hydrochloride therapy.

2. Materials and Methods

   2.1. Materials

Chitosan, sodium tripolyphosphate, phosphate-buffered saline, acetic acid, Cytophaga Agar, De-ionized water Oxytetracycline hydrochloride (purity 99%) and distilled water. All other chemicals were obtained from commercially from AL-Bashir Scientific Bureau/Iraq. Flavobacterium columnare isolate were taken from Department of Pathology/Fish Diseases College of Veterinary Medicine/Baghdad University.

2.2. Flavobacterium columnare

Isolate of F. columnare bacteria was taken from “Department of fish diseases College of Veterinary Medicine/Baghdad University”, the bacteria was a three-time passage into a stable common carp to improve bacterial virulence. Then, all biochemical tests were done to ensure the characteristics of the bacteria.
2.3. Preparation and loaded the chitosan nanoparticles

The chitosan nanoparticles were synthesized from the chitosan via sodium tripolyphosphate as a cross-linking agent by ionotropic gelation method (Vimal et al., 2013). About 1.5 g of chitosan dissolved in 200 ml of 2% acetic acid, this solution was placed for about 20 minutes under the magnetic stirrer process. Next to the above-initialised chitosan solution, (0.8 g) of sodium tripolyphosphate dissolved in (107 ml) of conductivity water were added drop wise. Then stirred well enough to reach equilibrium for around 30 minutes. A milky colored emulsion of chitosan nanoparticles appears, which was formed upon the ionic cross-linking between the sodium tripolyphosphate and chitosan solution. After achieving equilibrium, the suspension was established in the conditions mentioned above.

Oxytetracycline hydrochloride melted in distilled water was added to the ChNPs solution in the ratio of 1:1, under stirring for twenty-minute. In addition, this suspension then was left under ultrasonication for 45 minutes. After that lastly stirring for another, twenty minutes, to gain a final concentration of antibiotics 3.75 mg/ml (Du et al., 2009; Jain and Banerjee, 2008).

2.4. Experimental Design

This research was carried out at the (College of Veterinary Medicine of Baghdad, an Ichthyology laboratory). A total of 160 healthy carp fish selected and treated by the formalin at the concentration of (25ml/200L) water used for ten min to annihilate the fungal, bacterial, and parasitic infections. After that distributed into 16 glass aquaria filled with chlorine-free tap water and supplied with an air pump at a rate of 10 fish per aquarium (two replicates treatment) were maintained for each of the 7 treatments (T0, T1, T2, T3, T4, T5, Control(-) and Control (+). Then, 2 weeks of fish adaptation until beginning the experiment, and feeding on a commercial diet, at a rate of 2% of their body weight.

The bacterial concentration of *F. columnare* that used for infection test after a serial dilution was 0.2ml (1.8 × 10⁶ CFU/ml), then removal a few of scales near the caudal fin and injection subcutaneously. In this experiment some of infected did not eat feed and to ensure that all fish are treated, the immersion bath was used for treatment according to researchers (Jeff et al 2008; Julinta et al 2017). The trial injected fish treated by ordinary oxytetracycline hydrochloride as T0 in dose 20 mg/L and T1, T2, T3, T4 and T5 treated by chitosan nanoparticles loaded oxytetracycline hydrochloride in dose 20, 15, 10, 5, 2.5 mg/L respectively.

All the treatments given the bath treatment 1-hour per-day for 7 days, mortality was already recorded daily for up to 14 days throughout all treatments, and assessment of survival rate after 7 days of treatment end, bacteria recovered from blood and kidney of the experimental challenge fish were identified as *F. columnare* after re-isolated it. In all treatments, live and dead fish were examined for abnormal behaviours’ including movement, swimming and external lesions on the skin and gills. At the final of the experimental procedure, the fish were counted according to (Amend, 1981) to assess the survival rate.

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\text{Survival rate} \% = \frac{\text{No. of fish counted after 7 days post-treatment end}}{\text{No. of total fish counted at the initial time}} \times 100
\]

2.5. Blood collection and sample preparation

Blood samples were collected using 3 ml plastic syringe (G 23 da). Three randomly selected fish were picked per aquarium from caudal vessels. To prevent any blood clot, the syringe was flushed with heparin. No anaesthetic medications have been used to avoid any possible effect on blood content, and the handling time was less than 1 minute to reduce the stress. The first part of the blood was transferred to lithium-heparin coated sterile Eppendorf tubes, which are used to measure blood haematology.
The second part was transferred to the gelatin-containing Eppendorf tubes and permitted to clot for 2 hours. Serum was already separated via centrifugation, store at -20°C that can be used for biochemical parameters (Albumin, Globulin and Albumin/Globulin Ratio).

2.6. Determination of Haematological Parameters:

2.6.1. Determination of Total Erythrocytes and Leukocytes Counts:

The total of erythrocyte and leukocyte count through diluted 20 μl of the freshly obtained blood sample with (0.98 ml) of Dacie solution. (10ml of 40% formaldehyde, 31.3g trisodium citrate, 1.0 g brilliant crystal blue dissolved in one L of distilled water and filtered through 0.45 μm syringe filter). Counts were performed on an improved hemocytometer of Neubauer. A drop in the hemocytometer was dripped, five squares were used to count the number of red blood cells (Dacie and Lewis, 1984). The followed equation was applied:
Total RBCs (Erythrocyte / μL6) = N (number of cell) x 2500

WBC’s were counted in four squares (four corner squares) (Dacie and Lewis, 1984). The following equation was applied:
Total WBCs (Leukocyte / μL3) = N (number of cell) x 125

2.6.2. Packed Cell Volume (PCV %)

Haematocrit (packed cell volume) of total blood was calculated using the capillary system, the heparinized blood was drawn by heparinized capillary tube and one of its ends blocked with sealing material. Next separated using a haematocrit centrifuge (5000 round/min for 5 minutes, following centrifugation, the total percentage of PCV was determined with haematocrit reader (Thrall et al., 2006).

2.6.3. Haemoglobin (Hb) content

The cyanomethemoglobin process (Blaxhall and Daisley, 1973) was used to assess haemoglobin amounts (Hb). By using haemoglobin kit, the haemoglobin reaction with cyanide was dependent upon for the formation of cyanomethemoglobin. The test tube administered with 20 μl non-coagulant blood that had been freshly obtained, applied to the 5 ml diluents mix (Drabkin's reagent), and left at room temperature for 5-10 minutes (25°C). Absorbance or optical density (O.D) was measured using a spectrophotometer at 540 nm The Drabkin Diluents reactant (1.0 g of sodium bicarbonate, 50 mg of potassium cyanide, 200 mg of potassium ferricyanide, dissolved in 1L water), was a stock of cyanomethemoglobin as normal, distilled into a multi-dilutant (Dacie and Lewis, 1995).

3. Results

3.1. Treatment and Survival rate of C. carpio:

Following the carp fish injected with F. columnare, the treatment of the infected fish was started when mortality appeared in the fourth day after bacterial injection. All the treatments are given the treatment 1-hour per-day for 7 days; mortality was recorded daily up to 14 days in the all groups and recording the survival rate after 7 days of treatment end, and observe the clinical signs of treated carp fish daily after the end of treatment. The results revealed a clear increase in survival rate in T4 that were 90% and T3 were 70% followed by T5 and T2 which give the same percentage 55% .while, T1 was recorded the lowest survival rate 40% as shown in the Table 4.6. In comparison with T0 (OTC-HCL), 20 mg/L was recorded low survival rate 60%. And Control + (no treated group) were recorded 15% survival rate.
Table 1. Survival and Mortality Rate of *Cyprinus carpio* treated with ChNP-OTC following challenge of *F. columnare*

| Treatment | Total number | No. of dead fish | Survival rate% | Mortality rate% |
|-----------|--------------|------------------|----------------|-----------------|
| Control(-) | 20           | 0                | 100            | 0               |
| Control(+) | 20           | 17               | 15             | 85              |
| T0        | 20           | 8                | 60             | 40              |
| T1        | 20           | 12               | 40             | 60              |
| T2        | 20           | 11               | 55             | 45              |
| T3        | 20           | 6                | 70             | 30              |
| T4        | 20           | 2                | 90             | 10              |
| T5        | 20           | 9                | 55             | 45              |

3.2. Clinical signs of *C. carpio* before and after treatment:

In the experimental infection test and pretreatment, the moribund fish showed off feed, swam on the surface of the water with increased operculum movement without reflecting the external impact and staying on the side before death. The gross symptoms involved a mild discoloration of the skin. Hemorrhagic swelling mainly at the injection site, gills also congested with necrotic lesion patches, Mortality of fish started after 4 days post-injection *F. columnare*. These symptoms are shown in figures (1. A, B, C&D).

One week after the end of the treatment period, an improvement in health was observed, as well as swimming and breathing also was regularly, especially in T4, T3, T5 and T0 also Control(-). Post-treatment the feed was given to the fish in all groups at the rate of 2%. No feed consumption in C+, T1 and T2. While in T4, T3, T5, T0 all the feed was consumed, and the fish were active and swim normally.

3.3. Hematological parameters:

3.3.1. Red blood cells count (RBC)
The results revealed significant increases (P<0.05) in RBCs count of *C. carpio* post-treatment in treated groups T4 (2.26 cell×10⁶/mm³) compared with control (+) group (1.40 cell×10⁶/mm³) and another treatment Table (4.8.1). However no significant difference (P 0.05) with control (-). While, treated groups T0, T1, T3 and T5 (1.61, 1.56, 1.73 and 1.56 cell×10⁶/mm³ respectively) were revealed slight increases and no significant difference (P 0.05) with C+ and between them, Table 2, and there were a significant decreases (P<0.05) in Control (+) compared with control (-).

3.3.2. Packed cell volume and Haemoglobin (PCV, Hb)

In the Table 2, there were significant increases (P<0.05) in concentration and packed cell volume and haemoglobin in treatments T3, T4 and T5. That were (20.43, 26.46 and 21.13 % respectively), and (7.31, 8.95, and 7.03 g/dl respectively) compared with Control (+) (15.95% and 5.94 g/dl) and recorded highest values in T4 from all other treatments which no and no significant difference (P > 0.05) with Control (-). In T0, T1, and T2 (19.40, 16.76, and 18.36% respectively) and (6.46, 5.59, and 6.07 g/dl respectively), that were no significant difference between them, but there is a significant increases (P<0.05) with Control (-), and there were a significant decreases (P<0.05) in Control (+) compared with Control (-).

3.3.3. White blood cells (WBC)

In Table 2, the results revealed no significant difference in WBCs count of *C. carpio* post-treatment in group T0 (19.26×10³ cell/mm³) compared with Control (-) and Control (+) (17.94 and 19.13×10³ cell/mm³ respectively). However, the recorded highest mean values and significant increases (P< 0.05) in T1, T2, T3, T4 and T5 (22.10, 23.36, 22.03, 21.00 and 20.50×10³ cell/mm³ respectively) in comparison with Control (-), but no significant difference (P 0.05) between them. Highest mean values in T1. These results mean WBC numbers showed a significant increase (P< 0.05) in treated groups.

### Table 2. Hematological parameters of *Cyprinus carpio* treated with ChNP-OTC following challenge of *F. columnare*

| Group       | Mean ± SE | RBC×10⁶/mm³ | PCV%      | Hb g/dl | WBC×10³/mm³ |
|-------------|-----------|-------------|-----------|---------|-------------|
| Control (-) | 2.13 ± 0.14 | 25.23 ± 0.14 a | 8.41 ± 0.04 a | 17.94 ± 0.67 d |
| Control (+) | 1.55 ± 0.03 b | 15.95 ± 0.43 e | 5.94 ± 0.47 de | 19.13 ± 1.04 cd |
| T0          | 1.61 ± 0.07 b | 19.40 ± 0.62 bc | 6.46 ± 0.21 cd | 19.26 ± 0.46 cd |
| T1          | 1.56 ± 0.08 b | 16.76 ± 0.17 cd | 5.59 ± 0.05 e | 23.36 ± 0.52 a |
| T2          | 1.76 ± 0.08 b | 18.36 ± 0.56 cd | 6.07 ± 0.24 de | 22.03 ± 0.40 ab |
| T3          | 1.73 ± 0.03 b | 20.43 ± 1.08 b | 7.31 ± 0.33 b | 21.00 ± 0.49 bc |
| T4          | 2.27 ± 0.12 a b | 26.46 ± 0.46 a | 8.95 ± 0.34 a | 22.10 ± 0.72 ab |
| T5          | 1.57 ± 0.08 b | 21.13 ± 0.77 b | 7.03 ± 0.26 bc | 20.50 ± 0.64 bc |

LSD value 0.275 * 1.821 * 0.841 ** 1.950 *

Means having with the different letters in same column differed significantly. * (P≤0.05)

3.3.4. Biochemical parameters
Results of total protein, albumin, globulin, A/G ratio

Total protein level revealed a significantly increased (P≤0.05), in treatments T2, T3, T4, and T5 (3.95, 4.25, 4.45, and 3.85 g/dl respectively) compared with Control (−) and Control (+) (2.60, 2.40 g/dl respectively). While, no important difference (P > 0.05) between them. In addition, the maximum levels were recorded in T4. Table 3. While the result of total protein in T0 and T1 (2.40 and 2.91 g/dl respectively) revealed no important difference (P > 0.05) with Control (−) and Control (+).

As well, Globulin level in this trial resulted in a significantly increased (P≤0.05), in treatments T2, T3, T4, and T5 (2.20, 2.30, 2.68, and 2.21 g/dl respectively) compared to the Control (−) and Control (+), (1.15, and 1.20 g/dl respectively). While, T0, T1 (1.24, and 1.63 g/dl respectively) revealed no significant difference (P > 0.05) compared with Control (−) and Control (+) and between them. The result of A/G ratio revealed a significantly decreased (P≤0.05) in all treatments in compared with Control (−) and no significant difference (P > 0.05) compared with Control (+). The best ratio recorded in T4 and T5 but not differ significantly from T3, T2 and T1 as shown in Table 3. There were a significant decreases (P<0.05) in Control (+) compared with Control (−).

| Group | Mean ± SE | Total protein g/dl | Albumin g/dl | Globulin g/dl | A/G |
|-------|-----------|-------------------|--------------|---------------|-----|
| Control (−) | 2.60 ± 0.18 cd | 1.45 ± 0.21 bcd | 1.15 ± 0.03 d | 1.23 ± 0.18 |
| Control (+) | 2.40 ± 0.10 d | 1.19 ± 0.01 d | 1.20 ± 0.11 cd | 0.996 ± 0.10 ab |
| T0 | 2.40 ± 0.10 d | 1.16 ± 0.06 d | 1.24 ± 0.16 cd | 0.963 ± 0.17 ab |
| T1 | 2.91 ± 0.23 c | 1.29 ± 0.08 cd | 1.63 ± 0.19 c | 0.803 ± 0.08 b |
| T2 | 3.95 ± 0.07 b | 1.75 ± 0.11 ab | 2.20 ± 0.05 b | 0.793 ± 0.06 b |
| T3 | 4.25 ± 0.03 ab | 1.95 ± 0.10 a | 2.30 ± 0.10 ab | 0.853 ± 0.08 b |
| T4 | 4.45 ± 0.16 a | 1.76 ± 0.06 ab | 2.68 ± 0.19 a | 0.663 ± 0.07 b |
| T5 | 3.85 ± 0.06 b | 1.57 ± 0.11 bc | 2.21 ± 0.19 b | 0.716 ± 0.10 b |
| LSD value | 0.408 * | 0.324 * | 0.428 * | 0.351 * |

Means having with the different letters in same column differed significantly. * (P≤0.05).

4. Discussion

The presence of skin lesion and gill necrosis are similar to characteristic lesions reported by Kubilay et al. (2008). Columnaris disease is primarily an epithelial infection that causes necrotic gill or skin lesions (Thune, 1993; Noga, 2000). Off feed and swim at the surface with increased operculum movement are observed by Tripathi, (2003). In addition, Tien et al. (2012) showed the infected striped catfish swim frequently with convulsions at the surface of the water, in particular, apparent in the first 24 hr post-bacterial exposure. The gills in these fish turn into a deep red color with alternate white patches. In addition to that, these fish had de-pigmented patches on the body, with a yellowish
discoloration on the fins. Finally, they had more severe tail erosion and the gills were fully necrotic. Between 48-52 h the mortalities happened after exposure to experimental bacterial immersion. In this study, the possible application of synthetic ChNPs for OTC administration in carp fish was evaluated. To date, there is a powerful need to develop new and alternative administration processes for this antibiotic in the aquaculture sector due to its wide use (Rigos and Smith, 2015). Exposure to ChNPs-OTC did not cause any important stress response in treated fish, that agreement with Chemello et al. (2015) when utilizing them the Oxytetracycline delivery in adult female Zebrafish by iron oxide nanoparticles.

The results of this study revealed a significant increase in survival rate in T4 and T3 and mortality rates decreased from 85% in the infected non-treated group to 10 and 30% in these immersion-treated groups, respectively. There are no similar studies. While, Rach et al., (2008) in the channel catfish trial, survival at (10 days) post-treatment was remarkably (P > 0.05) greater for all Oxytetracycline-hydrochloric treatment groups comparative to controls, and the Oxytetracycline-hydrochloric treatments effectively decreased mortality in walleyes (10 mg/L only) and channel catfish infected with F. columnare.

Another study, Julinta et al., (2017) evaluated the effectiveness of oxytetracycline (OTC) oral and bath therapies on Nile tilapia Oreochromis niloticus against A. hydrophila infection, where they are found. The oral and bath therapies in fries with OTC recorded low mortalities (17 and 21%) than the respective control. In this study we comparison with some studies of oxytetracycline effect of the fishes, also effects of chitosan nanoparticles studies compared to ChNP-OTC that due to no similar comparison studies in vivo. Hematological parameters have been recognized as valuable tools for assessing the health status of fish. These parameters are affected by several factors, such as species of fish, age, period of sexual maturity and health status (Blaxhall, 1972; Hrubec et al., 2001).

Changes in physicochemical parameters may be reflected in blood parameters of the fishes (Abdul Naveen et al., 2011). The results of this study revealed a significant decrease in RBCs count, Hb content and PCV% in non-treated Control compared with control group, but there is slight increase in WBC value in Control than control. All of that agreement with (Tripathi et al., 2005), when they registered considerable changes in blood parameters were noticed in the infected koi carp fish. For the hematologic parameters, a remarkable reduction was observed in hemoglobin concentration, Packed Cell Volume (PCV), and red blood cell count. From our observation on all treatments there is increase the RBC count value in all treated groups compared with no treated group (Control). In addition, the higher value in T4 which has low dosage of ChNP-OTC 5mg/L where, recorded the less stress during treatment period on carp fish. That agreement with (Chemello et al., 2016) where they was recorded the exposure of Oxytetracycline Delivery in Adult Female Zebra fish by Iron Oxide Nanoparticles fish to the complex resulted in a 10 times higher OTC accumulation with respect to using water exposure. In addition, in fish exposed to 4 mg/L OTC (using water), the liver antibiotic concentration was very low during the completely experimental time.

Also agreement with (Nikapitiya et al., 2018) where was recorded Chitosan nanoparticles: a positive immune response modulator as display in zebra fish larvae against Aeromonas Hydrophila infection and CNPs exposure at 5 μg/mL could enhance the immune responses and develop the disease resistance against A. hydrophila, which could be attributed to its strong immune modulatory properties. The result of PCV and Hb value in our study showed decrease significantly (P < 0.05) in higher concentration treatment T0, T1, and T2. This decrease is compatible with previous studies showed that OTC can be responsible for the induction of toxicity in fish liver by altering the activity of enzymes involved in stress response (Bruno, D. W. 1989; Pari and Gnanasoundari, 2006; Nakano et al., 2018). Also, PCV and Hb value in T4 recorded increase significantly (P < 0.05) compared with all treatments.

In this study, the WBC count showed increase significantly in T0, T1, T2, T3 and T5 compared with Control. While in T4, the deference is insignificantly. Increase in number of WBC in diseased fish could be serving as a protective barrier towards any infection (Talpur and Ikhwanuddin, 2013). Moreover, WBCs are one of the most affecting factors in immunity of fish and WBC counts
has been used as marker of health of aquatic animals (Duncan and Klesius, 1996). That depend on 
range of immune response against bacteria. The antibiotic generally inhibits growth of the pathogen 
allowing the immune system to eliminate the invaded microorganisms and chitosan nanoparticles and 
its loaded antibiotics kill and inhibits the growth of bacteria due to the antibacterial activity increased 
with increasing the antibiotic content, that agreement with (Ibrahim et al., 2015)

In the study by Sebastião et al.,(2011) they have recorded the blood parameter results from 
naturally infected tilapia displayed that there were changes in the erythrocytic series and in organic 
defense blood cells, in the fish infected with the bacterium, with a decrease in erythrocytic variables 
and important raise in the numbers of neutrophils and circulating lymphocytes. The total serum protein 
appeared the most significant indicator of the nutritional state of the health condition of fish (Patriche, 
2011). Other researchers notified that the total protein concentrations, albumin, and globulin in the 
plasma is an indicator of liver function and subsequently the reduction of serum protein may be 
referred to renal excretion or impaired protein synthesis, or because of the liver hypofunction or 
disorder (Bernet et al., 2001).

In this study, Total proteins, Albumin and Globulin levels increased significantly in 
treatments (T2, T3, T4, and T5), that indicates an improvement of innate immunity for fish. This result 
is in line with Ranjan et al. (2014) in Asian seabass (Latescalcarifer). Similar findings recorded in 
olive flounder Cha et al. (2008), the rising level of total serum protein may be caused by the activity of 
bactericidal, activity of serum lysozyme, globulin content and perhaps a few other peptides (Misra et 
al., 2006).

5. Conclusions

The present study represents an important first step for the study of the effect of chitosan 
nanoparticles loaded with oxytetracycline hydrochloride on the health status of common carp with 
Columnaris disease. As a result, this new drug delivery method appears to be much better in relation to 
the conventional method of antibiotic exposure, as it reduces the dose of antibiotics and increases the 
survival rates against bacterial infection in fish, and reducing the antibiotic-resistant fish, and reduces 
the potential environmental impacts. However, the action of chitosan nanoparticles-loaded 
oxytetracycline is unclear and more studies are needed to understand its mechanism of action and side 
effects on fish industry.

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