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## **Ameliorative Efficacy of Garlic Extract on Erythromycin and Copper Sulfate Induced Side Effects in Nile Catfish**

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## SUMMARY

Aquaculture is currently considered the largest single source of fish supply in Egypt. Where, fish is one of the animal origin proteins, which is characterized by their content of high quality animal protein, minerals, vitamins, essential amino acids and omega-3 fatty acids.

The present study was conducted to throw light on the possible drastic effects of erythromycin (macrolide antibiotic) and copper sulfate (disinfectant) on hematological, some immunological, some biochemical parameters in Nile catfish (*Clarias gariepinus*) and their histopathological changes in liver, kidneys and gills. Moreover, attempting to ameliorate these possible side effects in fish, this study was also undertaken to determine the efficacy of garlic extract (*Allium sativum* L) as an alternative to antibiotics (to decrease resistance to erythromycin by *Pseudomonas aeruginosa*) and disinfectants commonly used in Nile catfish aquaculture. As well as, to evaluate garlic immunostimulatory effect on *C. gariepinus* disease resistance to *Pseudomonas aeruginosa*.

This study was held on a total number of 210 healthy *Clarais gariepinus* with an average body weight of (150±5 gm), which were randomly allocated into 7 equal groups (each

of 30 fish) which divided in 3 replicates per group and each replicate contains 10 fish as the following:

**Group 1 (G1) (Control group):** Fish of this group were served as control non treated which were fed on basal diet only.

**Group 2 (G2) (treated with erythromycin):** Fish were fed on basal diet and treated with erythromycin (50 mg/kg b.wt.) for 7 successive days then change water and leaving fish without medication till the end of the experiment.

**Group 3 (G3) (treated with copper sulfate):** Fish were fed on basal diet and treated with copper sulfate (2 mg/l) for each 10 successive days with 10 days rest between each treatment along the experimental period (8 weeks).

**Group 4 (G4) (treated with erythromycin and garlic):** Fish were fed on basal diet supplemented with garlic (10 gm/kg diet) and treated with erythromycin (50 mg/kg b.wt.) for the same regime of treatment in (G2).

**Group 5 (G5) (treated with copper sulfate and garlic):** Fish were fed on basal diet supplemented with garlic (10 gm/kg diet) and treated with copper sulfate (2 mg/l) for the same regime of treatment in (G3).

**Group 6 (G6) (treated with 10 gm garlic):** Fish were fed on basal diet supplemented with garlic (10 gm/kg diet) for 8 weeks.

**Group 7 (G7) (treated with 20gm garlic):** Fish were fed on basal diet supplemented with garlic (20 gm/kg diet) for 8 weeks.

Three blood samples were collected from the caudal blood vessels of each group on 10<sup>th</sup> day, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks from beginning of the experiment

**a) Sample (1):**

The first blood sample was collected from the tail blood vessels in clean sterilized tubes containing an anticoagulant (EDTA) for hematological examination.

**b) Sample (2):**

In plain centrifuge tubes, blood sample was collected from the tail blood vessels without an anticoagulant. The sample was allowed to coagulate, and then the serum was separated by centrifugation at 3000 r.p.m for 10 minutes for serum separation and stored at - 20 °C in sterile Eppendorf tubes until used for estimation of serum nitric oxide, lysozyme activity, serum IgM level, total serum protein, albumin, globulins and the percentage of each fraction (albumin, alpha, beta and gamma-globulins) by electrophoresis.

**c) Sample (3):**

In heparinized syringe, blood sample was collected for determination of phagocytic percent and phagocytic index.

On 10<sup>th</sup> day, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks from beginning of the experiment, 3 fish per aquarium were randomly sampled, sacrificed with a blow to the head. Specimens from liver, kidneys and gills were collected to be used for studying the histopathological changes.

At the end of experimental period (8 weeks), The fish from treated groups and control one (20 fish/group) were clinically examined and blood samples bacteriologically tested and determined to be free from bacterial infection and then challenged with 0.5 ml of *Pseudomonas aeruginosa* ( $1 \times 10^7$ ) intra peritoneal (I/P). Fish were kept under observation for recording the clinical signs and mortality rate up to 15 days post challenge. Specimens from liver, kidneys and gills were collected at the end of 15 days post challenge for histopathological examination.

**The results of this investigation can be summarized into the following:**

**A) Effect on some hematological parameters:**

Most of hematological parameters (including: RBCs, Hb, PCV%, MCV, MCHC) and leukogram parameters (WBCs,

heterophils, lymphocytes and monocytes) were significantly decreased in fish groups treated with either erythromycin or copper sulfate. Thus, leukopenia, lymphocytopenia and heterophilia is the main picture of the leukogram for *C. gariepinus* of this groups comparing control non treated group. While, all these parameters were relatively improved in the garlic-supplemented groups and this improvement was increased with the duration of feeding trial.

#### **B) Effect on some immunological parameters:**

The immunological parameter (phagocytic activity, nitric oxide, lysozyme activity and IgM level) of *Clarias gariepinus* that treated with either erythromycin or copper sulfate were significantly decreased when compared with control non treated group. Therefore, administration of garlic to fish diet significantly improved all these parameters and there is positive correlation between the immunological status improvement and the duration of the feeding regime.

#### **C) Effect on some serological parameters:**

Fish treated with either erythromycin or copper sulfate displayed significant high ALT, AST and ALP (indicating liver functions failure) and high urea and creatinine activity (indicating renal failure). Those fish also displayed significant

decrease in serum total protein, albumin, globulins and its fractions. But, the addition of garlic succeeded to ameliorate the elevation in ALT, AST, ALP, urea and creatinine. Also, showed significant increase in serum total protein, albumin and globulin compared with erythromycin and copper sulfate exposed fish groups. These results give an obvious indication about the role of garlic in the maintenance of the integrity of liver and kidney morphology.

#### **D) Challenge test:**

The results of the challenge test by *Pseudomonas aeruginosa* after 8 weeks of experimental treatment trials were indicated that the usage of garlic powder in *C. gariepinus* feeding diet either alone or in combination with erythromycin or copper sulfate were led to good protection from the disease caused by pathogenic *Pseudomonas aeruginosa* strain that was proved by clinical picture and mortality rate.

#### **E) Histopathological findings:**

The results revealed certain severe changes affecting the parenchymatous organs (liver, kidneys and gills) of *Clarias gariepinus* induced by therapeutic doses of erythromycin and copper sulfate. Moreover, these changes were more observed in copper sulfate treated group (G3).

Erythromycin and copper sulfate treated groups in combination with garlic supplemented diet (10gm/kg) showed mild to moderate observations with nearly normal appearance of majority of hepatic, renal and gills structures especially with the progress of experimental period where garlic extract could ameliorate the drastic effect of erythromycin and copper sulfate on histological findings. The histopathological lesions nearly disappeared at the end of the experimental period.

Histopathological examination of groups treated with different two doses of garlic revealed normal tissue architecture and cellular details with no remarkable pathological alterations of liver, kidneys and gills.

The results also were recorded an improvement in the histopathological findings in liver, kidneys and gills tissue in the challenged fish with *Pseudomonas aeruginosa* that were previously fed on garlic supplemented diet either alone or in combination with erythromycin or copper sulfate than the challenged fish that were fed on normal control diet either treated or non-treated.