

IMMUNOSTIMULATING EFFECT OF LEVAMISOLE ON NONSPECIFIC IMMUNITY OF AFRICAN CATFISH (CLARIAS GARIEPINUS) AFTER IMMUNOSUPPRESSION INDUCED BY MALATHION

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ABSTRACT

One hundred and sixty African catfish, obtained from Abbassa fish farm were distributed into four equal groups (40 each) in aerated glass aquaria (100 L capacity) and given balanced pellets at 2% of body weight twice daily. The fish were allowed 2 weeks for acclimatization. Gp. (1) was the control. Gps. (2-4) were kept in 0.01 ppm sublethal concentration of Malathion for one week. Gps. (3&4) were then given levamisole 2-hour bath at 2.5 and 5 mg/Liter respectively. Blood samples were collected from 5 fish (gps. 1-4) at 0, 7, 14 and 21 days after the application of levamisole to study nonspecific defense mechanisms (total and differential leukocytic count, macrophage chemotaxis, lymphocytic proliferation index, serum and mucus lysozymes and serum immunoelectrophoresis).

The total leukocyte and lymphocyte counts, macrophage chemotaxis and globulin were significantly increased from the 1st to 3rd weeks of levamisole treatment when compared with gp. (2). The serum and mucus lysozyme and lymphocyte blast transformation were significantly increased from the 1st to 2nd weeks only when compared with gp. (2). These results indicate that levamisole is effective in augmenting the non-specific defense system.

INTRODUCTION

Great attention all over the world, specially in the developing countries including Egypt, has been directed towards the supply of edible proteins, rich or at least sufficient in the essential amino acids, that are not easily synthesized in the mammalian body. The amino acid pattern of fish is closely related to that found in the animal protein, so fish is an essential source of cheap

animal protein facing the continuous increase of population.

Investigations on the effects of environmental contamination on fish health have increased in recent years. In particular, chronic exposure to sublethal concentrations of heavy metals, pesticide or other chemicals has been suspected of increasing the sensitivity of fish to infectious diseases (**Siwicki and Studnicka, 1992**). Therefore, the study of sublethal and chronic effects of pollutants on the immune system in fish has become important.

Organophosphorus insecticides are widely used in agriculture, and in fisheries for the control of plankton invertebrates and for the treatment and prevention of parasite infestation. Previous studies on the effects of organophosphorus compounds on fish health have concentrated on factors affecting their toxicity to fish, and the kinetics of elimination from fish and the determination of withdrawal periods (**Saudinka and Sopinska, 1983; Jeney and Jeney, 1986 and Demael et al., 1990**).

Malathion is the organophosphorous insecticide most frequently used on crops and fields as an insecticide in addition to pest control in agriculture area in Egypt. Malathion has been known to cause a specific inhibition of acetylcholinesterase, which in some cases is accompanied, by the inhibition of neuron target esterase (**Repetto et al., 1988**). Malathion depressed the nonspecific immune response of fish (**Siwicki and Studnicka, 1992**).

Studies on human and animals have demonstrated the effect of levamisole on the activity of immune function. Levamisole has been documented as potential immunostimulator; thus is used in the treatment and prophylaxis of fish diseases. **Siwicki (1987 and 1989)** recorded enhancement of phagocytic activity, leukocyte migration, myeloperoxidase activity and increased lysozyme levels in fish treated with levamisole. Application of levamisole in food or bath enhanced the phagocytic activity of neutrophils and macrophages in fish (**Siwicki, 1989; Siwicki et al, 1989 and Findlay and Munday, 2000**). An in vitro study showed an immunostimulatory effect of levamisole on the lymphocyte proliferation, macrophage and neutrophils activity in carp and rainbow trout (**Siwicki and Cossarini-Dunier, 1990 and Siwicki et al., 1992**).

Findlay and Munday (2000) reported the first record of the use and efficacy of levamisole as an immunomodulator in Atlantic salmon fish, however there is no available literature about the effect of levamisole as immunostimulant in catfish *Clarias gariepinus*. This study was performed to investigate the immunomodulation effect of levamisole on the nonspecific immunity in African catfish *Clarias gariepinus* after immunosuppression induced by Malathion.

MATERIAL AND METHODS

Fish :

One hundred and sixty African *Catfish Clarias gariepinus* weighing 150-200 gm were transferred from Abbassa fish farm to the laboratory where they were acclimatized in a 100-L glass aquaria filled with dechlorinated tap-water, supplied with continuous aeration for 2 weeks for acclimatization. Fish were fed twice daily with nutritionally balanced pellets at 2% of body weight.

Experimental Design :

The fish were divided into four equal groups (40 each). Gp. (1) acted as a control. Gps. (2-4) were subjected to 0.01 ppm sublethal concentration of Malathion for one week (LC₅₀ of Malathion in catfish *Clarias gariepinus* body weight range 90-120 gm was 0.044 ppm by *El-Bagori, 2000*). Gps. (3&4) were then treated with levamisole 2-hours bath at a rate of 2.5 and 5 mg/Liter respectively. Following bath, all groups were held in full sanitary fresh-water system. Blood samples were collected from 5 fish of each group at 0, 7, 14 and 21 days after the first application of levamisole to study the nonspecific defense mechanisms.

Assay of nonspecific defenses

The total and differential leukocytic counts, macrophage chemotaxis, lymphocytic proliferation index, serum and mucus-lysozymes and serum immunoelectrophoresis were determined.

The total and differential leukocytic counts were determined according to (**Stoskoph, 1993**). Peritoneal macrophage chemotaxis were assayed according to the method described by **Klesius and Sealey (1996)**. The lymphocytic proliferation index was determined according to **Ota (1984)**. Serum and mucus-lysozymes were determined turbidimetric assay according to **Sankaran and Gurnani (1972)**. Immunoelectrophoresis of serum protein was done using cellulose acetate according to (**Henry et al., 1974**).

Statistical Analysis:

The data were analyzed by analysis of variance (ANOVA) using **State View 4.01 (1993)** followed by Dun's multiple range test to indicate the groups, which were significantly different at ($P < 0.05$).

RESULTS & DISCUSSION

The results from gps. (3&4) were closely similar. Our study revealed increased nonspecific immune response in levamisole treated fish (tables, 1, 2 & 3).

The leukogram profile showed an increase in both the total leukocyte and lymphocyte counts in levamisole treated fish at the end of the 1st to 3rd weeks when compared with Malathion group, and at the end of the 2nd week only when compared with the control group (table, 1).

The macrophage chemotaxis assay gps. (3&4) revealed a significant increase from the end of the 1st to 3rd weeks when compared with gp. (2) and from the end of the 2nd week when compared with the control group (table, 2).

There was a significant increase in the lymphocyte transformation index (gps. 3&4) at the end of the 1st and 2nd weeks when compared with gp. (2), and at the end of the 2nd week only when compared with the control group (table, 2).

The serum and mucus (gps. 3&4) displayed a significantly increased lysozyme activity when compared with gp. (2) at the end of the 1st and 2nd weeks and at the end of the 2nd week only when compared with the control group (table2).

The serum electrophoresis patterns showed that the γ -globulin (gps. 3&4) was significantly increased from the 1st to 3rd week in comparison with gp. (2) and at the end of the 2nd week when compared with the control group (table, 3).

The specific immune response takes days to develop because it involves complex pathways of selection and synthesis of specific molecules such as antibodies. However the nonspecific defense mechanisms are ready and often need to be activated (Douglas et al., 1991). Thus the nonspecific defense mechanism reacts faster after immunization, injury or infection by microorganisms. Then nonspecific defense mechanism assays, done with fish, include demonstrating activities of phagocytosis and chemotaxis of macrophages and monocytes (Weeks and Warinner, 1986).

An alternative approach to vaccines and antibiotics would be the use of immunostimulating agents. During the last decade there has been an increasing interest in the modulation of the nonspecific immune system of fish for treatment and prophylaxis measure against diseases. Immunostimulants, as levamisole, have shown to be powerful activators of nonspecific defense mechanism which is the first line of defense against microbial infections, thereby preventing infectious diseases (Sahoo and Mukherjee, 2001 & 2002).

Granulocytes and mononuclear phagocytes play a central role in the cell-mediated immunity of the nonspecific defense of fish (Dalmo et al., 1996). The progressive increase of the total leu-

kocytic count in levamisole treated groups could be a result of stimulation of leukopoiesis by levamisole. Our results are in-agreement with **Siwicki and Studnicka (1992)** who reported elevated total leukocytic count in *Cyprinus carpio* administered 5 mg/Kg.B.W. every three days for 28 days after induced immunosuppression with trichlorfon pesticide. On the other hand **Findlay and Munday (2000)** reported insignificant differences in leucocrit value of levamisole treated fish with 2.5 mg/L for 2 hours when compared with the control group.

Lymphocyte blast transformation is a relatively simple, rapid and reproducible test, utilized in clinical and experimental immunology (**Ota, 1984**). Lymphocyte transformation is the means for monitoring the effect of immunoenhancing or suppressive therapy where lymphocyte transformation may be defective even without detectable lymphopenia (**Lopez et al., 1975**). Our study shows that levamisole treated fish showed a significantly augmented proliferation of T-lymphocyte. Levamisole as an immunomodulating of lymphocyte transformation has been documented with **Hajzic et al., (1999)** and **Abdalla et al., (1995)** in children, suffering brain tumor and pre or postoperative medication patients respectively. **Johnkoski et al., (1996)** attributed the increased proliferation of mice T-lymphocyte treated with levamisole to enhanced interleukine-6 production with T-lymphocytes. Meanwhile **Cabaj et al., (1995)** recorded a decrease in lymphocyte transformation in lambs drenched with levamisole.

Macrophages represent the host's second line of defense to infectious agents, therefore determination of macrophage functions including chemotaxis is important in the evaluation and diagnosis of the defense disorder pathways (**Ammann and Fudenberg, 1980**). Fish macrophages have potent bactericidal and larvicidal activities, in addition to possessing both intracellular and extracellular killing mechanisms (**Secombes and Fletcher, 1992**). Our work revealed an increased macrophage chemotaxis response in immunocompromised fish treated with levamisole. **Sahoo and Mukherjee (2001)** recorded that feeding of levamisole to immunocompromised *Labeo rohita* fish, with aflatoxin, could restore neutrophil oxidative radical release and phagocytic activity. Similarly the macrophage phagocytic ability has been enhanced in Atlantic salmon fish treated with levamisole (**Findlay and Munday, 2000**).

Lysozymes are widespread enzymes with antibacterial role, in many teleost tissues and secretions (**Lindsay, 1986**). The lysozymes are distributed in fish tissues rich in leukocytes, and at sites where the risk of invasion is high (skin, gills and gastrointestinal tract) where lysozymes provide a protective function against viruses, neoplasms, bacteria, fungi and insects (**Dobson et al., 1984**). In the recent years there has been an increase in the number of studies reflecting the importance of lysozymes as nonspecific immune system in fish. Our studies confirm the efficacy of levamisole in increasing the activities of both serum and mucus lysozymes. **Findlay and Munday (2000)** recorded a significant increase in both mucus and serum lysozymes in Atlantic sal-

mon fish after 2-weeks of levamisole bath 2.5 mg/L for 2-hours. Also **Sahoo and Mukherjee (2001)** reported elevated serum lysozyme activities in *Labeo rohita* fish fed on dietary intake of levamisole (5mg/ kg body weight at 3 day interval for 60 days).

Although the electrophoresis technique did not directly detect the immune defects, it is valuable and relatively simple technique that may help the diagnosis of a possible immunologic defect, also it is a valuable tool in assessing humoral immunity and in differential diagnosis (**Kaneko et al., 1997**). Our studies revealed that the levamisole restored the total protein and gamma-globulin levels in the immunosuppressed fish. Similarly **Sahoo and Mukherjee (2001 and 2002)** reported increased total protein, albumin, globulin and antibody titer level by feeding levamisole to immunosuppressed fish with aflatoxin B1. In contrary **Morrison et al. (2000)** mentioned that the levamisole as adjuvant has a narrow range of efficacy when used by bath or intraperitoneal injection in Atlantic salmon fish.

As a point of view levamisole is potent immunostimulant with both doses 2.5 and 5.0 mg/Liter through enhancement of the nonspecific immune system. This finding agrees with **Ziberg et al., (2000)** who recorded insignificant difference of immune response in *Salmo salar* fish exposed to 1.25, 2.5 and 5.0 mg/liter levamisole.

This study provides strong evidence that bath treatment with levamisole enhances the non-specific immune system. Also the present results suggest that the bath treatment of immunocompromised fish with levamisole may increase their resistance to infection, reduce fish mortality and could be of economic benefit.

Table (1): Total and differential leukocytic count in African catfish *Clarias gariepinus* treated with levamisole after immunosuppression induced by Malathion.

| Group | 1 (Control) a | | | | | | 2 (Malathion) b | | | | | | 3 (Levamisole) c | | | | | | 4 (Levamisole) d | | | | | |
|--------|---------------|-------|------|------|------|------|-----------------|-------|------|------|------|------|------------------|-------|------|------|------|------|------------------|-------|------|------|------|------|
| | TLC | Lymp | Neut | Esin | Baso | Mono | TLC | Lymp | Neut | Esin | Baso | Mono | TLC | Lymp | Neut | Esin | Baso | Mono | TLC | Lymp | Neut | Esin | Baso | Mono |
| 0 day | 25.24 | 15.41 | 8.02 | 0.36 | 0.01 | 1.44 | 19.99 | 9.71 | 8.69 | 0.31 | 0 | 1.28 | 19.53 | 9.25 | 8.62 | 0.34 | 0.01 | 1.31 | 19.71 | 9.31 | 8.58 | 0.43 | 0.01 | 1.38 |
| | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 7 day | 26.64 | 15.92 | 8.79 | 0.41 | 0 | 1.52 | 20.16 | 9.91 | 8.65 | 0.34 | 0.02 | 1.24 | 25.70 | 15.12 | 8.79 | 0.38 | 0 | 1.41 | 26.10 | 15.31 | 8.83 | 0.44 | 0.01 | 1.51 |
| | ± | ± | ± | ± | 0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | 0 | ± | ± | ± | ± | ± | ± | ± |
| 14 day | 25.46 | 15.18 | 8.41 | 0.39 | 0 | 1.48 | 20.4 | 10.28 | 8.45 | 0.36 | 0.01 | 1.34 | 33.37 | 22.94 | 8.51 | 0.44 | 0 | 1.48 | 34.38 | 23.75 | 8.65 | 0.49 | 0 | 1.49 |
| | ± | ± | ± | ± | 0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | 0 | ± | ± | ± | ± | ± | 0 | ± |
| 21 day | 25.86 | 15.28 | 8.59 | 0.47 | 0.01 | 1.51 | 23.55 | 13.01 | 8.61 | 0.44 | 0 | 1.49 | 29.00 | 18.42 | 8.58 | 0.45 | 0.01 | 1.54 | 29.07 | 18.51 | 8.62 | 0.48 | 0.01 | 1.45 |
| | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 2.41 | 1.83 | 0.81 | 0.04 | 0.01 | 0.16 | 2.27cd | 1.42 | 0.85 | 0.03 | 0 | 0.12 | 2.62b | 1.92b | 0.87 | 0.03 | 0.01 | 0.19 | 2.65b | 1.95b | 0.75 | 0.04 | 0.01 | 0.15 |

Significant at a, b, c, d < 0.05

- TLC = Total leukocyte count.
- Lymp = Lymphocyte.
- Neut = Neutrophil.
- Esin = Eosinophil.
- Baso = Basophil
- Mono = Monocyte

Table (2): Macrophage chemotaxis, lymphocyte transformation and serum and mucus lysozymes in African catfish, *Clarias gariepinus*, treated with levamisole after immunosuppression by Malathion.

| Group | 1 (Control) a | | | | 2 (Malathion) b | | | | 3 (Levamisole) c | | | | 4 (Levamisole) d | | | | | |
|--------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---------------------|----------------------|----------------------|----------------------|---------------------|----------------------|----------------------|----------------|--|
| | Macrophage | | Lymphoc. | | Lysozyme µg/ml | | Macrophage | | Lymphoc. | | Lysozyme µg/ml | | Macrophage | | Lymphoc. | | Lysozyme µg/ml | |
| | Chemotaxis | Transform. | Serum | Mucus | Chemotaxis | Transform. | Serum | Mucus | Chemotaxis | Transform. | Serum | Mucus | Chemotaxis | Transform. | Serum | Mucus | | |
| 0 day | 9.91 ± 0.59bcd | 1.22 ± 0.06bcd | 6.89 ± 0.81bcd | 14.4 ± 1.21bcd | 5.81 ± 0.42a | 0.82 ± 0.01a | 3.89 ± 0.41a | 8.14 ± 1.11a | 5.92 ± 0.52a | 0.84 ± 0.02a | 3.48 ± 0.44a | 8.49 ± 1.28a | 5.84 ± 0.51a | 0.85 ± 0.02a | 3.45 ± 0.42a | 8.46 ± 1.27a | | |
| 7 day | 9.28 ± 0.51b | 1.21 ± 0.05b | 6.24 ± 0.57b | 15.3 ± 1.28b | 6.15 ± 0.49acd | 0.88 ± 0.02acd | 4.41 ± 0.40acd | 9.48 ± 1.13acd | 9.54 ± 0.54b | 1.37 ± 0.06b | 6.85 ± 0.42b | 15.91 ± 1.35b | 9.39 ± 0.52b | 1.32 ± 0.05b | 6.78 ± 0.41b | 15.74 ± 1.32b | | |
| 14 day | 9.45 ± 0.58cd | 1.11 ± 0.04cd | 6.45 ± 0.64cd | 15.89 ± 1.35cd | 7.01 ± 0.59cd | 1.01 ± 0.05cd | 5.74 ± 0.68cd | 13.18 ± 1.41cd | 14.21 ± 0.74ab | 1.68 ± 0.07ab | 10.81 ± 0.88ab | 21.46 ± 1.85ab | 14.53 ± 0.76ab | 1.62 ± 0.06ab | 10.91 ± 0.89ab | 22.51 ± 1.91ab | | |
| 21 day | 9.86 ± 0.61 | 1.19 ± 0.06cd | 7.82 ± 0.78 | 15.74 ± 1.32 | 8.04 ± 0.69cd | 1.12 ± 0.05 | 6.21 ± 0.67cd | 13.86 ± 1.48cd | 12.49 ± 0.88b | 1.31 ± 0.04 | 9.78 ± 0.69b | 17.12 ± 1.82 | 12.41 ± 0.79b | 1.34 ± 0.05 | 9.81 ± 0.74b | 17.01 ± 1.79 | | |

Significant at a, b, c, d < 0.05

Table (3): Serum electrophoresis of African catfish *Clarias gariepinus* treated with levamisole after immunosuppression induced by Malathion.

| Group | 1 (Control) a | | | | | 2 (Malathion) b | | | | | 3 (Levamisole) c | | | | | 4 (Levamisole) d | | | | |
|--------|----------------------|-------------------|-------------------|-------------------|----------------------|--------------------|-------------------|-------------------|-------------------|----------------------|--------------------|-------------------|-------------------|-------------------|---------------------|--------------------|-------------------|-------------------|-------------------|---------------------|
| | TP | Album | α glob | β glob | γ glob | TP | Album | α glob | β glob | γ glob | TP | Album | α glob | β glob | γ glob | TP | Album | α glob | β glob | γ glob |
| 0 day | 4.35 ± 0.22bcd | 1.42 ± 0.19 | 0.98 ± 0.15 | 1.46 ± 0.15 | 0.49 ± 0.05bcd | 3.19 ± 0.20a | 1.11 ± 0.18 | 0.77 ± 0.14 | 1.09 ± 0.18 | 0.22 ± 0.03a | 3.16 ± 0.22a | 1.09 ± 0.19 | 0.72 ± 0.15 | 1.15 ± 0.16 | 0.20 ± 0.02a | 3.12 ± 0.21a | 1.08 ± 0.18 | 0.74 ± 0.16 | 1.12 ± 0.15 | 0.18 ± 0.02a |
| 7 day | 4.39 ± 0.23b | 1.49 ± 0.17 | 0.96 ± 0.17 | 1.42 ± 0.16 | 0.52 ± 0.04b | 3.38 ± 0.19a | 1.18 ± 0.15 | 0.82 ± 0.15 | 1.13 ± 0.17 | 0.25 ± 0.02acd | 3.70 ± 0.24 | 1.26 ± 0.14 | 0.79 ± 0.14 | 1.19 ± 0.15 | 0.46 ± 0.03b | 3.74 ± 0.25 | 1.28 ± 0.15 | 0.80 ± 0.14 | 1.17 ± 0.16 | 0.49 ± 0.04b |
| 14 day | 4.41 ± 0.25 | 1.47 ± 0.18 | 0.99 ± 0.14 | 1.48 ± 0.18 | 0.47 ± 0.03bcd | 3.55 ± 0.25 | 1.23 ± 0.14 | 0.84 ± 0.13 | 1.21 ± 0.17 | 0.27 ± 0.03acd | 4.01 ± 0.21 | 1.29 ± 0.14 | 0.83 ± 0.15 | 1.20 ± 0.14 | 0.60 ± 0.04ab | 4.1 ± 0.22 | 1.31 ± 0.15 | 0.86 ± 0.16 | 1.26 ± 0.18 | 0.67 ± 0.04ab |
| 21 day | 4.39 ± 0.21 | 1.48 ± 0.18 | 0.96 ± 0.16 | 1.44 ± 0.17 | 0.51 ± 0.03 | 3.81 ± 0.22 | 1.29 ± 0.15 | 0.88 ± 0.15 | 1.28 ± 0.15 | 0.36 ± 0.04acd | 4.12 ± 0.25 | 1.36 ± 0.16 | 0.87 ± 0.16 | 1.31 ± 0.15 | 0.58 ± 0.04b | 4.14 ± 0.24 | 1.32 ± 0.15 | 0.88 ± 0.16 | 1.35 ± 0.18 | 0.59 ± 0.04b |

Significant at a, b, c, d < 0.05

TP = Total Protein

Album = Albumin

glob = globulin

REFERENCES

- Abdalla E. E., Adam I. J., Blair G. E., Boylston A., Sue-Ling H. M. and Finan P. (1995)** : The immunomodulatory effect of levamisole is influenced by postoperative changes and type of lymphocyte stimulant. *Johnston D Cancer Immunol Immunother.* 41(3):193-8.
- Ammann A. J. and Fudenberg H. H. (1980)** : Immunodeficiency Diseases, pp. 409-401. In basic and clinical immunology. Fudenberg H. H., Sites DP and Caldwell JL (eds). Lang Medical Publication, Canada.
- Cabaj W., Stankiewicz M., Jonas W. E. and Moore L. G. (1995)** : Levamisole and its influence on the immune response of lambs. *Vet Res Commun* 19(1):17-26.
- Dalmo R. A., Bogwald J., Ingebrigtsen K. and Seljelid R (1996)** : The immunomodulatory effect of laminaran(B 1,3D-Glucan) on Atlantic salmon, *Salmo salar*, anterior kidney leukocytes after intraperitoneal, preoral and preanal administration. *Journal of Fish Diseases.* 19, 449-457.
- Demacl A., Dunier M. and Siwicki A. K. (1990)** : Some effects of dichlorvos on carp metabolism. *Comparative Biochemistry and Physiology*,93: 237-240.
- Dobson D. E., Prager E. M. and Wilson A. C. (1984)** : Stomach lysozymes of ruminants. I. Distribution and catalytic properties. *Journal of Biological Chemistry.* 259, 11607-11613.
- Douglas P. A., Tadaaki M. and Ricardo G. (1991)**: Neutrophil, glass-adherent, nitroblue tetrazolium assay gives early indication of immunization effectiveness in rainbow trout. Elsevier Science Publishers. Amsterdam.
- El-Bagori H. M. (2000)** : Pathological studies on some environmental pollutants on some freshwater fish in Sharkia governorate. M. V. Sc. Thesis, Pathology Dept., Fac. Vet. Med., Zagazig Univ.
- Findlay V. L. and Munday B. L. (2000)** : The immunomodulatory effects of levamisole on the nonspecific immune system of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases.* 2, 369-378.
- Hajnzic T., Kastelan M., Lukac J. and Hajnzic T. (1999)** : Immunocompetent cells and lymphocyte reactivity to mitogens in levamisole-treated brain tumor children. *Pediatr Hematol. Oncol.* 16(4):335-40.
- Henry R. J., Cannon D. C. and Winkelman J. W. (1974)** : Clinical Chemistry: Principles and Techniques. P 473, Harper and Row. Hagerstown.
- Jeney Z. and Jeney G. (1986)** : Studies on the effect of trichlorfon on different biochemical and

physiological parameters of common carp (*Cyprinus carpio*). *Aquacult. Hungarica*. 5: 79-89.

Johnkoski J. A., Peterson S. M., Doerr R. J. and Cohen S. A. (1996) : Levamisole regulates the proliferation of murine liver T-cells through Kupffer-cell-derived cytokines. *Cancer Immunol Immunother* .43(5):299-306.

Kaneko, J. J., John, W. H. and Michael, L. L. B. (1997) : *Clinical Biochemistry of Domestic Animals*. 5 th Ed., Academic Press, San Diego, London, Tokyo and Toronto.

Klesius P. H. and Sealey W. M. (1996) : Chemotactic and chemokinetic response of channel catfish macrophages to exoantigen from *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*. 8: 314-318.

Lindsay, G. J. H. (1986) : The significance of chitinolytic enzymes and lysozymes in rainbow trout (*Salmo gairdneri*) defense. *Aquaculture* 51, 19-175.

Lopez C., Simons R. L., Touraine J. L. and Good R. A. (1975) : Discrepancy between PHA responsiveness and quantitative estimates of T cell numbers during chronic renal failure and immunosuppression after transplantation. *Clinical Immunology and Immunopathology*. 4: 135-142.

Morrison R. N., Nowak B. F. and Carson J. (2000) : The effect of levamisole as an adjuvant on the humoral immune response of Atlantic salmon (*Salmo salar L.*). *Bull. Of the European Ass. Of Fish Pathology.*, Vol. 20 (3) 101-105.

Ota B. (1984) : *Laboratory Technique of Veterinary Clinical Immunology*. Publisher Springfield, Illinois, USA.

Repetto G., Sanz P. and Repetto M. (1988) : In vivo and in vitro effect of trichlorfon on esterases of the red crayfish (*Procambarus clarkii*). *Bulletin Environmental Contamination and Toxicology*. 41: 16-22.

Sahoo P. K. and Mukherjee S. C. (2001) : Dietary intake of levamisole improves non-specific immunity and disease resistance of healthy and aflatoxin- induced immunocompromised rohu, *Labeo rohita*. *Journal of Applied Aquaculture*. 11 (4) 15-25.

Sahoo P. K. and Mukherjee S. C. (2002) : The effect of dietary immunomodulation upon *Edwardsiella tarda* vaccination in healthy and immunocompromised Indian major carp (*Labeo rohita*). *Fish and Shellfish Immunology*. 12, (1) 1-16.

Sankaran K. and Gurnani S. (1972) : On the variation in the catalytic activity of the lysozyme in fish. *Indian Journal of Biochemistry and Biophysics* 9, 162-165.

- Saudinka M. and Sopinska A. (1983)** : Toxicity of technical foschlor for fish. *Rocznik Nauk Rol.* 2: 111-116.
- Secombes C. J. and Fletcher T. C. (1992)** : The role of phagocytes in the protective mechanisms of fish. In: *Annual Review of Fish Diseases.* 2: 53-71.
- Siwicki A. K. (1987)** : Immunomodulating activity of levamisole in carp spawners (*Cyprinus carpio*). *Journal Fish Biology Suppl. A,* 31: 245-246.
- Siwicki A. K. (1989)** : Immunostimulating influence of levamisole on non-specific immunity in carp (*Cyprinus carpio*). *Develop. Comparative Immunology.* 13: 87-96.
- Siwicki A. K., Anderson D. P. and Dixon O. W. (1989)** : Comparisons of non-specific and specific immunomodulating by oxolinic acid, oxytetracycline and levamisole in salmonids. *Veterinary Immunology and Immunopathology.* 23: 195-200.
- Siwicki A, Anderson D. P. and Dixon O. W. (1992)** : In vitro effect of levamisole on the neutrophil activity in rainbow trout (*Oncorhynchus mykiss*). *Arch. Immunol. Ther. Exp.* 40 (5-6): 253-256.
- Siwicki A. K. and Cossarini-Dunier (1990)** : Effect of levamisole on lymphocyte and macrophage activity in carp (*Cyprinus carpio*). *Ann. Rech. Vet.* 21:95.
- Siwicki A. K. and Studnicka M. (1992)** : Stimulation of nonspecific immunity after immunosuppression induced by chemical stress in carp (*Cyprinus carpio*). Edited By Muller R and Lloyd R: *Sublethal and chronic effects of pollutants on freshwater fish.* Published by Arrangement with the Food and Agriculture Organization of the united nations by Fishing News Books.
- State View (1993)** : Version 4.01. Abacaus Institute. Berkeley, California.
- Stoskopf M. (1993)** : *Fish Medicine.* PP,116,128,129.W.B. Saunders company.
- Weeks B. A. and Warinner J. E. (1986)** : Function evaluation of macrophages in fish from polluted esturay. *Veterinary Immunology and Immunopathology.* 12: 313-320.
- Ziberg D., Findaly V. L., Girling, P. and Munday B. L. (2000)** : Effects of treatment with levamisole and glucans on mortality rates in Atlantic salmon (*Salmo salar* L.) suffering from amoebic disease. *Bulletin of the European Association of Fish Pathologists.* 20 (1): 23-27.

الملخص العربى

تأثير الليفاميزول كمنشط للمناعة الغير متخصصة فى أسماك القرموط الإفريقى بعد تثبيط مناعتها بالملاثيون

المشركون فى البحث

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أجريت هذه الدراسة لتقييم كفاءة الليفاميزول كمنشط للمناعة فى سمكة القرموط الإفريقى بعد تثبيط مناعتها بالملاثيون. تم تقسيم الأسماك إلى أربعة مجاميع (٤٠ سمكة فى المجموعة) بعد أن تم أقلمتها تحت الظروف المعملية فى أحواض زجاجية سعة ١٠٠ لتر لمدة إسبوعين وتغذيتها بنسبة ٢٪ من وزن جسم السمكة، المجموعة الأولى كانت ضابطة أما المجموعات الثانية والثالثة والرابعة فقد تم تعريضهم للملاثيون بتركيز ١٠٠ ر. جزء فى المليون لمدة إسبوع ثم تغيير المياه وتعريض المجموعتين الثالثة والرابعة لحمام مائى لمادة ليفاميزول بتركيز ٢٥٠ ، ٥ ملجم / لتر على التوالى لمدة ساعتين.

أظهرت النتائج ما يلى :-

- إرتفاع معنوى فى عدد كرات الدم البيضاء والخلايا اللمفاوية فى الأسماك التى تم معاملتها بالليفاميزول مقارنة بمجموعة الملاثيون عند نهاية الإسبوع الأول إلى نهاية الثالث بينما كانت معنوية عند نهاية الإسبوع الثانى فقط بمقارنتها بالمجموعة الضابطة.
- أوضحت نتائج الانجذاب الكيميائى للخلايا الأكلة للأسماك التى تم معاملتها بالليفاميزول إرتفاعاً معنوياً من نهاية الإسبوع الأول إلى نهاية الثالث بالمقارنة بمجموعة الملاثيون، فى حين كانت معنوية فى نهاية الإسبوع الثانى بالمقارنة بمجموعة الكنترول.
- وجد أن هناك زيادة معنوية فى معامل تحويل الخلايا اللمفاوية للأسماك التى حصلت على حمام مائى بالليفاميزول فى نهاية الإسبوع الأول والثانى بالمقارنة بمجموعة الملاثيون بينما كانت معنوية فى نهاية الإسبوع الثانى بمقارنتها بالكنترول.
- أظهرت النتائج أن هناك فروق معنوية فى نتائج إختبار المواد المحللة فى المصل والمخاط بالنسبة للأسماك التى تم

معالجتها بالليفاميزول عن المجموعات الأخرى.

- إرتفاع معنوى ملحوظ فى الجاما جلوبيولين للأسماك المعالجة بالليفاميزول عند نهاية الإسبوع الأول إلى نهاية الثالث بالمقارنة بمجموعة الملائيون وعند الثانى فقط بالمقارنة بالكنترول.

ويتضح من هذه الدراسة أن الليفاميزول له تأثير قوى وفعال فى رفع المناعة لدى أسماك القرموط الإفريقى وذلك للوقاية من الأمراض التى تصيبها وعوامل الإجهاد المختلفة.