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Research Article
Immunomodulatory Effects of Curcumin on Nile Tilapia, *Oreochromis niloticus* and its Antimicrobial Properties against *Vibrio alginolyticus*

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Abstract
The effect of dietary curcumin on the non-specific immune defence mechanisms and resistance against challenge with pathogenic *Vibrio alginolyticus* in Nile tilapia, *Oreochromis niloticus,* was evaluated. Fish were divided into two groups before being fed for 30 days with commercial diets supplemented with 0% (control) and 2% curcumin (weight/weight) at the rate of 2% of body weight. Fish were challenged with virulent *V. alginolyticus* on the 30 days of feeding experiment. Immune parameters including; peroxidase, serum bactericidal activity and serum proteins were investigated to analyze fish immune defence mechanisms. All immune profiles were significantly enhanced in fish fed with curcumin supplemented diets ($p<0.05$) compared to the control group. Challenge study indicated highest survivability (100%) in the group of fish fed with curcumin whereas, others fed the basal diet showed a survival rate of 40%. The immunomodulatory and antimicrobial effects of curcumin were evaluated also *in vitro* by assaying Nitric Oxide (NO) production via macrophages and well diffusion method, respectively. A gradual significant increase in nitric oxide production in parallel with higher curcumin dose was demonstrated. The uppermost NO levels, were detected in 50 µg concentration. All curcumin concentrations showed varying degrees of inhibition against tested *V. alginolyticus* strains and the minimal inhibitory concentration was determined as 12.5 mg mL^{-1}. Therefore, results confirmed substantial evidence to suggest that low concentrations of curcumin as food supplements are able to enhance the immune defence mechanisms of Nile tilapia and could be of value in protection against invading pathogens.

Key words: Curcumin, immunostimulation, antimicrobial effects, *Oreochromis niloticus, Vibrio alginolyticus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The global trend of commercial aquaculture is towards intensification of culture practice in the aim to increase productivity per unit area (Elgendy *et al*., 2015a). Consequently, fish vulnerability to infectious agents is boosted with major stock mortalities and substantial economic losses (Elgendy *et al*., 2015b, c; Moustafa *et al*., 2015).

Therefore improvement and innovation of secure methods to combat microbial infections in aquaculture is a critical concern. Traditional approaches include application of chemotherapeutics and antibiotics but the consequential ample undesirable environmental outcomes restrict their therapeutic purposes (Grigorakis and Rigos, 2011). On the other hand, efficient vaccines are not developed against all fish pathogens and their application is quite pricey (Vetvicka *et al*., 2013).

Strengthening fish defense mechanisms through prophylactic administration of immunostimulants has long been considered a stronghold in the control and management of diseases in aquaculture (Dugenci *et al*., 2003; Galina *et al*., 2009). Among the renowned category of such immunostimulants, medicinal herbs, rank first due to their broad spectrum activity, cost effectiveness and environmental-friendly disease preventative measures (Alishahi and Nejad, 2012).

Curcumin is the major biologically active component of turmeric, *Curcuma longa* and has long been reported to have diverse medicinal properties (Moghadamtousi *et al*., 2014) including; anti-inflammatory (Bereswill *et al*., 2010), antioxidant (Al-Jassabi *et al*., 2012), antimicrobial (Tajbakhsh *et al*., 2008) and antiviral activities (Kutluay *et al*., 2008) and immunomodulatory effects (Gupta *et al*., 2013). Additionally, curcumin has been found to lack significant toxicity in humans even at high oral doses up to 12 g per day (Cheng *et al*., 2001). Curcumin has been successfully used to control some bacterial infections in farmed fish like *Aeromonas hydrophila* in goldfish (Harikrishnan and Balasundaram, 2008) and *Vibrio harveyi* in black tiger shrimp, *Penaeus monodon* (Vanichkul *et al*., 2007).

Studies discuss the immunomodulatory effects of dietary curcumin in tilapia fish are scanty. Therefore the present study initiated to investigate such effects on Nile tilapia, *Oreochromis niloticus* and the potential resistance against challenge with pathogenic *V. alginolyticus* strain. Further the study aimed to evaluate the immunomodulatory and antimicrobial effects of curcumin *in vitro* with the determination of the Minimum Inhibitory Concentration (MIC).

MATERIALS AND METHODS

Diet preparation: Curcumin powder was purchased from (Lobal Chemie). A commercial pellet diet was crushed, mixed with tap water containing 2% of curcumin powder (2/100 g of powder) and made again into pellets. Remade pellets were allowed to dry and stored at 4°C.

Fish and experimental design: Nile tilapia, *O. niloticus* with average weight of 45±5 g were obtained from a private fish farm at Kafer El-Sheikh governorate Egypt. Fish were transported alive to laboratory of Hydrobiology Department, National Research Centre, Egypt, before acclimatized for 15 days in glass aquaria (90×45×45 cm) supplied with chlorine free tap water under continuous aeration. The water was maintained at 25±2°C, pH (7.6), dissolved oxygen (6.6 mg L^{-1}) and ammonia (0.01-0.005 mg L^{-1}). The photoperiod was adjusted to 12 h light:12 h dark. Fish were fed on commercial diet 40% protein at the rate of 2% of body weight.

Fish were divided randomly into 2 groups each with 30 fish (10 per replicate) and fed for 30 days. First group fed with diet supplemented with 2% of curcumin while, other group with basal commercial diets as control.

Determination of immune parameters *in vivo*

Collection of blood and serum: By the end of feeding experiment, after 30 days, blood samples were obtained from the caudal vein of experimental fish within the two groups for serum analysis and leukocytes isolation. Firstly, fish were anesthetized with clove oil (Merck, Germany) at 50 μL L^{-1} of water. Blood was drawn via syringe without anticoagulant for serum collection, tubes were kept in slanting position for about 2 h and thereafter centrifuged at 1600xg for 25 min at 4°C, followed by collection of straw colored serum with micropipette and stored at -20°C for further analysis.

Isolation of leukocytes: Leukocytes were isolated from blood according to (Rowley, 1990; Jeney *et al*., 1997). Briefly, 1 mL of histopaque 1.119 (Sigma, St., Louis, MO) containing bacto hemagglutination buffer, pH 7.3 was dispensed into a siliconized centrifuge tube. Then 1 mL of fish blood was carefully layered on top. The gradient was centrifuged at 500xg for 15 min at 4°C. Interface of leukocyte suspension was gently collected with a Pasteur pipette and dispensed into a siliconized tube before washed twice with phenol red-free Hank' s balanced salt solution (HBSS, Sigma) and adjusted to 2×10^6 viable cells per milliliter.

Peroxidase assay: The total peroxidase content in the serum was measured according to Quade and Roth (1997). About 50 µL of different sera obtained from the experimental fish groups was diluted with 135 µL of Hank's Balanced Salt Solution (HBSS) free from Ca^{2+} or Mg^{2+} in 96 well plates. About 25 mL of substrate buffer (Himedia, India) were added. The reaction was stopped after 2 min by adding 50 µL of 4 M sulphuric acid (H_2SO_4). Plate was c entrifuged at 400xg for 10 min and 150 µL of the supernatants, present in each well, were transferred to new 96 well plates. The OD was recorded at 450 nm in a microplate reader ELx 800 UV (Bio-Tek).

Serum bactericidal activity: Bactericidal activity of sera collected from fish was estimated following the procedure of Kajita *et al*. (1990). An equal volume (100 µL) of bacterial suspension and serum was mixed and incubated for 1 h at 25°C. Control sample was prepared by replacing fish serum with sterile PBS. The mixture was then diluted with sterile PBS at a ratio 1:10 then 100 µL of the mixture was pour-plated in nutrient agar and incubated for 24 h at 37°C. Bacterial colonies grown in the nutrient agar plates were counted to determine the number of viable bacteria.

Serum proteins: Different sera collected from fish groups were analysed for total protein and albumin content following the methods adopted from Doumas *et al*. (1971) and Lowry *et al*. (1951) respectively. Furthermore, globulin content was calculated by subtracting albumin from the total protein then albumin: globulin ratio was determined.

Challenge test: On the 30 days of feeding experiment, each fish within the two groups were intraperitoneally challenged with 0.2 mL of (3×10^7 CFU) culture suspension of pathogenic *V. alginolyticus* according to Elgendy (2007). This pathogenic bacterial strain was isolated previously from massive tilapia mortalities by our lab team (Hydrobiology Department National Research Centre, Egypt). Reisolation of injected bacteria from all freshly dead fish during the period of observation was carried out.

The total peroxidase content, serum bactericidal activity and proteins were also determined in sera collected from tilapia fish after the 2 weeks of challenge experiment following the previous described protocols.

***In vitro* Nitric Oxide (NO) assay:** Macrophages were derived from both the head kidneys and peripheral blood according to Soto *et al*. (2010) and Rajaraman *et al*. (1998) respectively, then cells were assayed *in vitro* for Nitric Oxide (NO) production following methods described by Tafalla and Novoa (2000) with some modification. Briefly, after incubation of macrophages the medium was aspirated from the plates. Fifty microliters of Hank's Balanced Salt Solution (HBSS) were added to each well with 50 µL of DMSO in triplicate manner as standard. The two collected macrophage groups were assayed, blood derived macrophages and the head kidney macrophages. Four different curcumin concentrations (150, 50, 15 and 1.5 µg) were added in triplicate manner. The plates were incubated for 2 h at 25°C in a humified 5% CO_2 atmosphere. Hundred microliters of the supernatants from each well was transferred into another plate and Hundred microliter of griess reagent were added. The mixtures were incubated at 21°C for 10 min. The Optical density was determined by universal microplate reader ELx 800 UV (Bio-Tek) at 540 NM. The molar concentration of nitrite in the sample was determined from standard curves previously generated with known concentrations of sodium nitrate.

***In vitro* antimicrobial effects of curcumin against *V. alginolyticus*:** The antibacterial activity of curcumin fraction was screened using the well diffusion method described by Perez *et al*. (1990). The *V. alginolyticus* reference strains were obtained from Hydrobiology department, Veterinary division, National Research Centre NRC, Egypt. Colonies of *V. alginolyticus* were transferred to sterile tubes each containing 5 mL of tryptic soy broth. Turbidity of the bacterial suspensions was adjusted to an optical density equivalent to a 0.5 McFarland standard to give a bacterial suspension of 10^8 CFU mL^{-1}: Colony Forming Unit.

Mueller-Hinton agar plates were inoculated via streaking bacterial swabs over the entire surface of the plates. Plates were then allowed to dry at room temperature and 6 mm wells were punched in each plate.

Three solutions of known curcumin concentrations were prepared by dissolving exact amount of curcumin fraction into measured volume of ethanol solvent (50, 100 and 400 mg mL^{-1}). Twenty microliters of each concentration were added into wells in each plate. The same amount of solvent, ethanol was also tested.

Plates were then allowed to stand at room temperature to let the tested derivative be diffused into the agar and afterwards, they were incubated at 37°C for 18-24 h, then examined for bacterial growth inhibition and zones of inhibition were measured in millimeters.

Standard streptomycin (30 µg $µL^{-1}$) and sulphamethoxizole with trimethoprim (1.25/23.75 µg) were used as positive control and sterile water as a negative control for comparison of the antibacterial activity. The experiment was carried out in triplicate and the mean value was recorded.

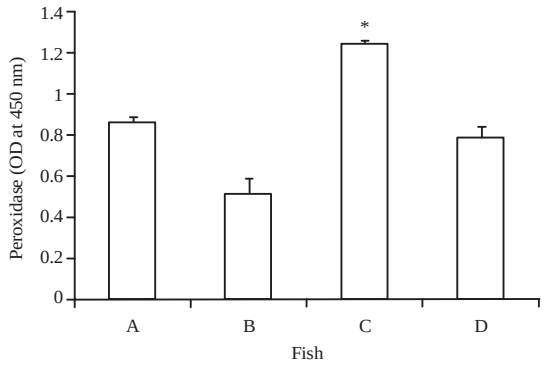

Fig. 1: Serum peroxidase activity of *O. niloticus* fed with curcumin supplemented diet and control basal diet, A: Control fish fed with basal diet, B: Control fish challenged with *V. alginolyticus*, C: Fish fed with curcumin supplemented diet and D: Fish fed with curcumin supplemented diet after challenge with *V. alginolyticus*. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

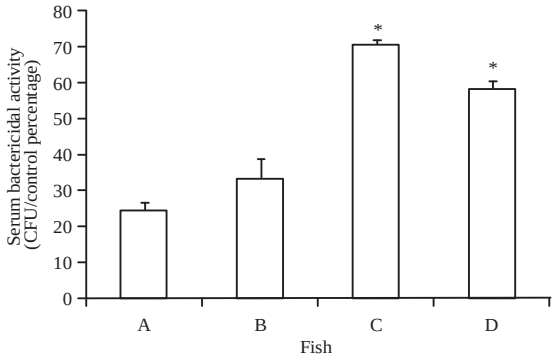

Fig. 2: Serum bactericidal activity of *O. niloticus* fed with curcumin supplemented diet and control basal diet, A: Control fish fed with basal diet, B: Control fish challenged with *V. alginolyticus*, C: Fish fed with curcumin supplemented diet and D: Fish fed with curcumin supplemented diet after challenge with *V. alginolyticus*. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

Determination of Minimum Inhibitory Concentration (MIC): Two-fold serial dilutions were prepared from curcumin solutions in tryptic soy broth. Duplicate tubes of each dilution were inoculated with bacterial strains. All tubes were incubated at 37°C for 18-24 h. The highest dilution of the curcumin that resulted in inhibition of bacterial growth was considered as the MIC.

Statistical analysis: Data were analysed by one-way analysis of variance (ANOVA). The differences among treatments were compared by Tukey's test using Minitab statistical software (Minitab, Coventry, UK). Differences were considered significant at $p<0.05$.

RESULTS

Effect of curcumin on peroxidase release via *O. niloticus* macrophages: The peroxidase activity of fish groups varied significantly in the two fish groups ($p<0.05$). The uppermost peroxidase content was detected in fish fed with curcumin supplemented diet (1.246±0.02) compared to control fish fed only the basal diet (0.860±0.03). Interestingly, after challenge with *V. alginolyticus*, peroxidase followed a decreasing trend but the curcumin group also showed relatively higher values (0.793±0.05) by comparison to the control group (0.507±0.08) as presented in Fig. 1.

Serum bactericidal activity: The serum bactericidal activity was significantly increased in tilapia fed with curcumin supplemented diet compared to control group including the post challenge period. The differences were significant ($p<0.05$) as compared with control. The highest amount of serum bactericidal activity was recorded on 30th day in the groups fed curcumin 70.41%±1.30 (CFU/control percentage) compared to control 24.83%±1.97 (CFU/control percentage). Bactericidal activity followed also an increasing trend in curcumin group 58.08%±2.38 after challenge with *V. alginolyticus* in comparison with fish fed with the basal diet 33.08%±5.71 (Fig. 2).

Serum proteins: The total protein, albumen and globulin increased significantly with the administration of curcumin in comparison with the control group including the post challenge period. On the contrary, the Albumin/Globulin (A/G) ratio was the lowest in the group of fish fed with curcumin (0.49±0.01) in comparison to others fed the basal diet (1.52±0.12) (Fig. 3-6).

Protection upon challenge: The survivability was found highest (100%) in the group fed with curcumin whereas the control group showed a survival rate of 40%. Furthermore, *V. alginolyticus* was re-isolation from all dead fish.

Nitric Oxide (NO) assay: The macrophage NO production was significantly enhanced after *in vitro* incubation of

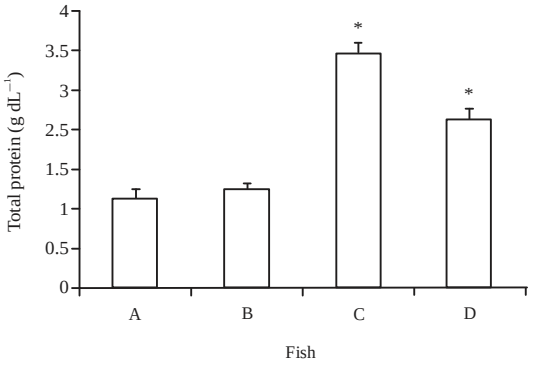

Fig. 3: Total serum protein of *O. niloticus* fed with curcumin supplemented diet and control basal diet A: Control Fish fed with basal diet, B: Control fish challenged with *V. alginolyticus*, C: Fish fed with curcumin supplemented diet and D: Fish fed with curcumin supplemented diet after challenge with *V. alginolyticus*. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

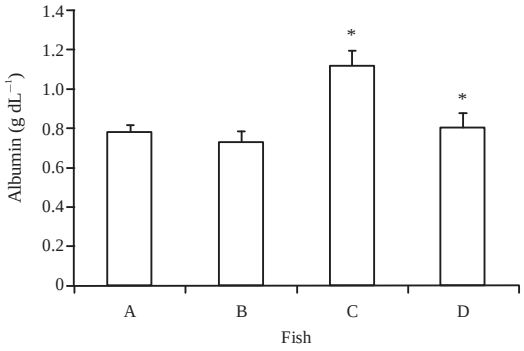

Fig. 4: Serum albumen of *O. niloticus* fed with curcumin supplemented diet and control basal diet, A: Control fish fed with basal diet, B: Control fish challenged with *V. alginolyticus*, C: Fish fed with curcumin supplemented diet and D: Fish fed with curcumin supplemented diet after challenge with *V. alginolyticus*. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

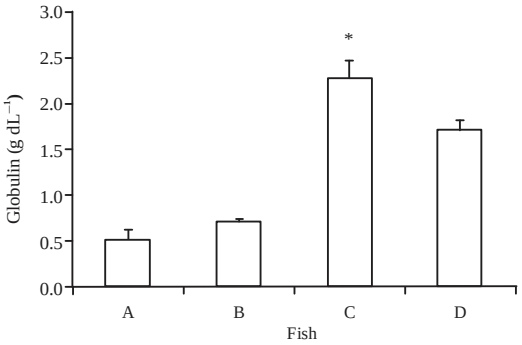

Fig. 5: Serum globulin of *O. niloticus* fed with curcumin supplemented diet and control basal diet, A: Control fish fed with basal diet, B: Control fish challenged with *V. alginolyticus*, C: Fish fed with curcumin supplemented diet and D: Fish fed with curcumin supplemented diet after challenge with *V. alginolyticus*. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

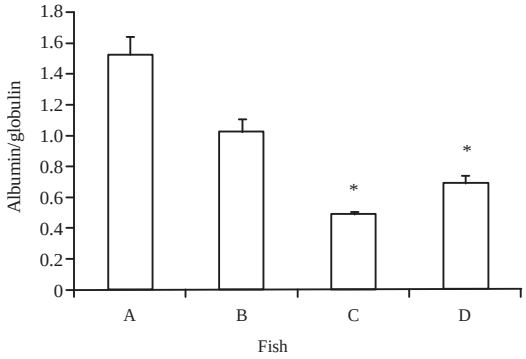

Fig. 6: Albumen/globulin ratio of *O. niloticus* fed with curcumin supplemented diet and control basal diet, A: Control fish fed with basal diet, B: Control fish challenged with *V. alginolyticus*, C: Fish fed with curcumin supplemented diet and D: Fish fed with curcumin supplemented diet after challenge with *V. alginolyticus*. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

macrophages with different curcumin concentrations; 150, 50, 15 and 1.5 μg. The NO production followed a gradual significant increasing trend parallel with higher crucumin concentration. The uppermost generation of NO was observed in 50 μg crucumin while, 150 μg achieved a non significant increase. Moreover, head kidneys derived macrophages demonstrated the highest values of NO production in comparison with peripheral blood macrophages (Fig. 7 and 8).

***In vitro* antimicrobial effects and minimum inhibition concentration:** All prepared curcumin concentrations (50, 100 and 400 mg mL^{-1}) showed varying degrees of inhibition against tested *V. alginolyticus* strains with zone of inhibition

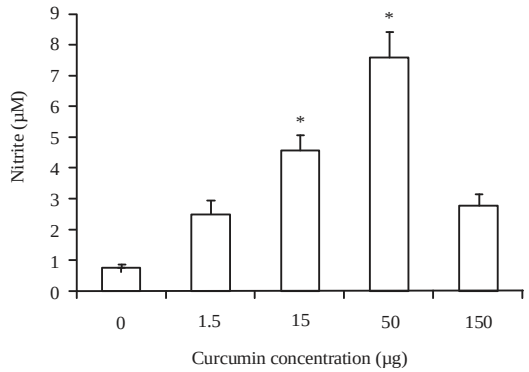

Fig. 7: Effect of different curcumin concentrations on nitric oxide production via head-kidney macrophages. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

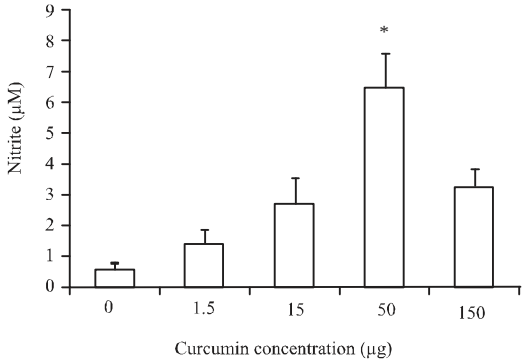

Fig. 8: Effect of different curcumin concentration on nitric oxide production via peripheral blood macrophages. Data are presented as Mean±SE. Asterisks represent significant difference from control ($p<0.05$), bars = Mean±SE

ranged as 13, 15 and 19 mm, respectively. The MIC was found to be ranging between 12.5 mg mL^{-1}. The results of the two reference antibiotics streptomycin and sulphamethoxizole/trimethoprim compound also showed activity against *V. alginolyticus* with inhibition zone of about 13 and 15 mm respectively on the other hand absolute ethanol and water were inactive (Fig. 9).

DISCUSSION

Interest in the application of immunostimulants as an alternative method to chemicals and antimicrobials currently being utilized to control fish diseases is growing. The use of medicinal plant products as potential therapeutic measures for modulating the immune mechanisms as well as to control

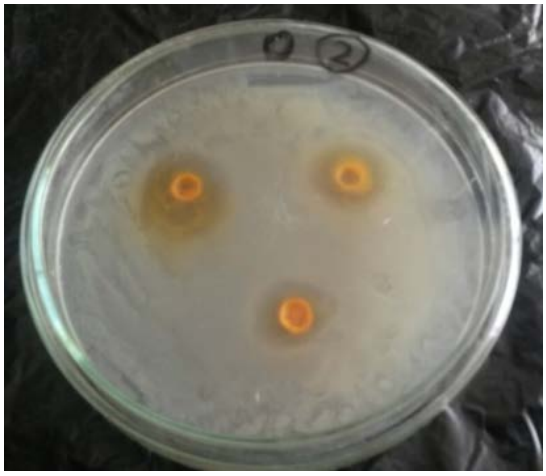

Fig. 9: *In vitro* antimicrobial effects of curcumin against *V. alginolyticus*

fish diseases have been focused on (Chakrabarti *et al*., 2014). Numerous herbs are presently used in commercial aquaculture as potent antimicrobial agents, nutrients as well as growth-promoting substances (Vaseeharan and Thaya, 2014; Yilmaz and Ergun, 2014).

Results demonstrated that peroxidase content was increased significantly in the group of fish fed with curcumin supplemented diet in comparison with control fish fed basal diet ($p<0.05$). The peroxidase produced by fish macrophages are alleged to play significant roles in innate immune-defenses mechanisms against various fish pathogens (Stosik *et al*., 2001) as well as has been considered as a reliable measure for neutrophilic antimicrobial activity particularly the primary granule exocytosis (Quade and Roth, 1997).

In the presence of halide and hydrogen peroxide, peroxidase generates oxidants destroy pathogens by halogenation of their cell wall (Fischer *et al*., 2006). The hypochlorous acid produced by peroxidase is a potent bactericidal (Klebanoff, 1967) and markedly potentiates the antibacterial potency of other reactive oxygen species involved in fish defenses (Rosen *et al*., 1990). Inadequate peroxidase activity in fish fed with the basal diet may indicate poor host defense mechanisms. In agreement with this study, enhancement of peroxidase activity in fish fed with different immunostimulant supplements have been reported likewise; *Achyranthes aspera* fed to *Labeo rohita*, *Cyprinus carpio* (Chakrabarti *et al*., 2012) and andrographolide in *Labeo rohita* (Basha *et al*., 2013).

The serum bactericidal activity was significantly increased in fish group fed with curcumin supplemented diet comparedto the control group including the post challenge

period ($p<0.05$). The bactericidal activity has been viewed as an important tool to analyze the innate immune system. It evaluates the presence of protective proteins in fish blood. Augmented serum bactericidal activities indicate elevated humoral factors involved in the innate and/or adaptive immune mechanisms (Wang *et al*., 2010). Diverse immunostimulants have been found to promote the serum bactericidal activity. *Epinphelus tauvina*, fed diets supplemented with extract mixture of some herbs; *Cynodon dactylon*, *Piper longum*, *Phyllanthus niruri*, *Tridax procumbens* and *Zingiber officinalis* showed a significant increase in their serum bactericidal activity (Punitha *et al*., 2008). Additionally, dietary administration of *Zingiber officinale* enhanced Indian major carp, *Catla catla*, serum bactericidal activities (Arulvasu *et al*., 2013).

The present results revealed significant increase in total protein, albumen and globulin levels in Nile tilapia fed with curcumin supplemented diet compared to control fish including the post challenge period ($p<0.05$). Similar results were detected in Indian major carp, *Catla catla*, on dietary administration of *Zingiber officinale* (Arulvasu *et al*., 2013) as well as in *Labeo rohita* fed with *Magnifera indica* kernel (Sahu *et al*., 2007).

Serum proteins are considered a fundamental index of fish health. Enhanced serum total proteins and globulin levels are thought to associate with stronger fish innate immune response mechanisms (Wiegertjes *et al*., 1996). Globulins in particular, play a significant role in the immune-protective mechanisms of fish (Sahoo and Mukherjee, 2001) and constitute integral part of almost all the immunoglobulins of blood (Misra *et al*., 2006).

The Albumin/Globulin (A/G) ratio has long been considered a reliable measure for the humoral components of nonspecific immune defenses (Misra *et al*., 2006). The low A/G ratio in curcumin treated group reflects overproduction of globulins, with significance for enhanced immune-protective mechanisms induced by the stimulatory effect of curcumin on fish non specific immune responses. On the other hand the high A/G ratio noticed in fish fed with the basal diet suggests underproduction of immunoglobulins. Similarly, feeding *Labeo rohita* fish with levan supplemented diets enhanced the non-specific cell mediated immune parameters and significantly reduced the A/G ratio (Gupta *et al*., 2008).

Survivability following challenge with *V. alginolyticus* was highest (100%) in fish fed with curcumin, whereas the control group showed a survival rate of 40%. Similarly, Nile tilapia fed a mixture of Astragalus and Lonicera extracts had an enhanced immune defence mechanisms as well as increased survival against challenge with *Aeromonas hydrophila* (Ardo *et al*., 2008). Moreover, intraperitoneal injection of *Oreochromis mossambicus* with water soluble fraction of *Solanum trilobatum* significantly enhanced production of reactive oxygen species and decreased fish mortality following a challenge with *A. hydrophila* (Divyagnaneswari *et al*., 2007).

Using fish primary macrophages as a model to investigate the dynamic response process of immunological parameters is highly valued. *In vitro* experiment demonstrated that NO production was significantly enhanced after incubation of macrophages exposed to heat killed *V. alginolyticus* with different curcumin concentrations; 150, 50, 15 and 1.5 µg. NO production followed a gradual significant increasing trend parallel with higher crucumin concentration. The uppermost synthesize of NO was observed in 50 µg crucumin while, 150 µg achieved a non significant increase. Moreover, head kidneys derived macrophages demonstrated the highest values of NO production in comparison with blood macrophages. Esteban and Meseguer (1997) have reported that defensive mechanisms of fish macrophages are functionally influenced by tissue source and variation is relevant to their maturation stage. Additionally, studies also demonstrated that monocytes freshly isolated from blood need a long to functionally mature (Shaala *et al*., 1979).

Diverse immune stimulating substances have been found to modulate the functions of macrophages *in vitro*. Exposure of rainbow trout, *Oncorhynchus mykiss*, macrophages retrieved from head kidneys and spleen with the immunomodulator muramyl dipeptide (MDP) *in vitro* resulted in a significant time-dependent accumulation of NO in cultures (Zvizdic *et al*., 2012).

Nitric oxides do many crucial roles in the immune defence mechanisms as well as having a direct antimicrobial effect (Villamil *et al*., 2002). The NOs are considered as potent free oxygen radicals and strongly involved in the pathways of a broad spectrum of diseases performing as a cytotoxic agent in pathological processes (Bogdan *et al*., 2000; Bogdan, 2000; Aktan, 2004). Furthermore, NOs can inhibit diversity of pathogens including, viruses, bacteria, parasites and many fungal infections (Yang *et al*., 2013). Additionally, NOs mediate the ability of macrophages to kill or inhibit the growth of many pathogens since it accumulates inside phagocytic vacuoles until it reaches the concentration required to produce the bactericidal effect (Bogdan, 2001).

Regarding the *in vitro* antimicrobial effects of curcumin against *V. alginolyticus*, prepared curcumin concentrations showed varying degrees of inhibition against tested *V. alginolyticus* strains and 12.5 mg mL^{-1} was identified as the MIC. The antimicrobial activities of curcumin and turmeric extracts against different bacteria have been reported in some

previous studies (Moghadamtousi *et al*., 2014). The *C. longa* was found to have inhibitory effect against some bacterial strains including; *A. hydrophila* (Harikrishnan and Balasundaram, 2008), *Helicobacter pylori* (Zaidi *et al*., 2009), *Pseudomonas aeruginosa* (Negi *et al*., 1999), *E. coli* (Gupta and Ravishankar, 2005), *Listeria monocytogenes, Salmonella typhimurium* and methicillin-resistant *Staphylococcus aureus* (Kim *et al*., 2005) as well as *K. pneumoniae* and *Staphylococcus epidermidis* (Niamsa and Sittiwet, 2009). The mechanisms of curcumin antimicrobial effects are relevant to suppression of bacterial cell proliferation (Rai *et al*., 2008) as well as disruption of prokaryotic cell division (Kaur *et al*., 2010).

CONCLUSION

Fish survival in the aquatic environment requires a competent immune system to overcome the constant challenge with pathogens. Results showed that curcumin can be a promising candidate for immunostimulation as well as a rival substitute for many antimicrobials currently used in tilapia fish farming. The activation of non-specific immune profiles in fish fed with curcumin supplemented diet has direct evidence o n improved health status of the host that might have maintained protection against challenge with *V. alginolyticus.* Prophylactic administration of dietary curcumin could be of value in protection against invading pathogens as well as minimizing mortalities stemming from disease outbreaks attacking farmed aquatic species.

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