SUMMARY AND CONCLUSION
In the present investigation, the affected gold fish was obtained from the fish rearing farms and microorganisms were isolated from the organs such as muscle, gill, liver and intestine. The highest microbial load ($6.3 \pm 0.4 \times 10^7$ cfu g$^{-1}$) was observed in muscle tissue of the infected $C.\ auratus$. The lowest microbial load ($4.3 \pm 0.7 \times 10^4$ cfu g$^{-1}$) was found in intestine of the fish $C.\ auratus$. The percentage distributions of mycotic and bacterial isolates are observed. The biochemical characterization of the pathogenic isolates was performed and the microorganisms were identified up to the generic level.

Based upon the antibiotic susceptibility test the five isolates such as $Escherichia\ coli$, $Aeromonas\ hydrophila$, $Staphylococcus\ aureus$, $Aeromonas\ salmonicida$ and $Vibrio$ sp. were selected and were administered to the healthy normal $Catla\ catla$ fish and LC$_{50}$ value was calculated. The mortality rates of $Catla\ catla$ exposed to different concentration of bacterial strains are evaluated. From the mortality rates, the LC$_{50}$ value was calculated and recorded. The LC$_{50}$ value for the $A.\ hydrophila$ was $5.4 \times 10^6$ CFU/ml, $A.\ salmonicida$ was $2.51 \times 10^6$ CFU/ml, $Vibrio$ spp., $2.81 \times 10^6$ CFU/ml, $Escherichia\ coli$ was $3.16 \times 10^6$ CFU/ml and $Staphylococcus\ aureus$ was $3.16 \times 10^6$ CFU/ml respectively.
For the pathogens isolated from the diseased fish *C. auratus* the antibiotic susceptibility test was performed. About 5 isolates showed 100% resistant to the antibiotics used.

Based on the results of lethal concentration and minimum inhibitory concentration test to the isolates, *A. hydrophila* was found to be highly antigenic to the fish *Catla catla* and hence *A. hydrophila* strain was selected for preparation of antigen.

From the *A. hydrophila* strain five different types of antigens such as heat killed antigen, whole cell antigen, heat killed antigen with antiserum, whole cell antigen with antiserum and nucleotide antigens were prepared and injected into the experimental fish (*Catla catla*) groups for the study of immunomodulation. Analysis of immunogenicity of antigens against the fish *Catla catla* was estimated. The *A. hydrophila* produced β hemolytic pattern on the blood agar plate.

B Lymphocytes counts using rosette forming assay revealed significant decrement in pathogens exposed fishes than in control.

Fishes exposed to pathogenic strains (1/10th sublethal concentration) for 3 weeks showed reduction in PFC. Effect of pathogenic antigens in direct splenic plaque forming cells (1g M producing cells) showed a reduction in secondary plaque forming cell in the first 3 weeks and a time and dose dependent decrease in primary and secondary PFC response.
A remarkable observation enhancement in B cell production is due to immune complex of antigens was noted in the present study. The enhancement of this type of immune responses confirms the potential of immune complexes to be used as vaccines.

‘T’ cell production of control and treated animals were estimated by rosette forming assay. The result showed significant changes in *Catla catla* fishes, when compared to control of five kinds of antigen treatment, the increment in ‘T’ lymphocyte number was much pronounced in *Catla catla* treated with heat killed antigen with antiserum.

The delayed type hypersensitivity reaction to tuberculin and DNCB antigen were tested in control and pathogen exposed fishes. The impact of pathogens on DTH response in fishes. The size of skin edema also declined in whole cell and heat killed antigen exposed fishes. The reduced development of skin reaction in fishes after the exposure to two antigens suggests possible impairment in the immune capacity of the fishes.

Among the different antigens exposed in fish, when compared with other antigens, the lymphocyte migration in fishes exposed to nucleotide antigen shows fastest migration. Lymphocyte migration was also significant in heat killed antiserum. Hence, the heat killed antiserum and nucleotide antiserum exposed fishes causes tolerance in immunity and provide defense against microbial infections and alterations.

The effects of heat killed antigen, vaccine, vaccine with adjuvant on the leucocyte count of fish *Catla catla* was studied. The differential counts of leucocytes such as lymphocytes, monocytes, eosinophils and basophils was determined and it was found to
be higher in the fishes treated with the vaccine and adjuvant than the control and fishes treated with heat killed antigen and vaccine alone. The results also imply that the lymphocytes are the most dominant leucocyte type in the blood of *Catla catla*.

Fish maintained as control showed normal structural organization of cells with granulated cytoplasm and uniform nuclei in liver. Fish treated with pathogen showed total loss of hepatic architecture with abnormal hepatocyte nuclei, hepatic necrosis, cytoplasmic vacuolization and conjunction of sinusoids. The liver shows a perivascular round-cell infiltration.

In fish maintained as control the lymphoid areas of spleen were found to be normal. In fish treated with antigen congestion and free apoptotic debris were observed in spleen. Histopathological analysis of spleen using light microscope revealed the aggregation of Melanomacrophage Centre (MMC) and production of mast cells in heat killed and live *A. hydrophila* induced experimental groups than that in control. The spleens show increased melanomacrophage activity and it is surrounded by a massive accumulation of round cells and fibroblasts. Small chains of coccoid bacteria are visible within this area of inflammatory cells.

In conclusion, the results from the present investigation suggest that it is impendingly achievable to develop a commercial vaccine against the *A. hydrophila* using immune complexes which will overcome the issues of the heterogenicity of the bacterium. It is also possible to improve the vaccine by adding additional antigens to other diseases to this formulation. The aquaculture desperately needs such a formulation to manage and overcome the distressing disease problem caused by the microorganisms.