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Prevention of histopathological damages in the liver, spleen and kidney of *Channa punctata* infected with *Aeromonas hydrophila.*

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ABSTRACT

It is now well established that chemical contaminants and biological pathogens deteriorate fish health and cause huge mortalities. Therefore, it is a challenge for the fisheries to produce large amount disease free food at low price. This study shows deteriorative changes in tissue of *Channa punctata* due to infection of *Aeromonas hydrophila*, a gram negative fish pathogenic bacterium. The effect of *Ficus benghalensis* aerial root and *Leucaena leucocephala* pod seed powder on vital organs of fish (liver, spleen & head kidney) of fish when challenged with *A. hydrophila* and fed with an artificial fish feed @ 5% w/w was studied. Fish were divided as: Group I) Negative control (injected with saline and fed on non-supplemented feed), Group II) Positive control (challenged with *A. hydrophila* and fed on non-supplemented feed), Group III & IV) Experimental groups challenged with *A. hydrophila* and fed on feeds supplemented with *F. benghalensis* and *L. leucocephala*, respectively. Histopathological examination of liver, spleen and kidney tissue of *C. punctata* from positive control group showed severe damage. While experimental groups III & IV did not show any considerable changes with respect to negative control group. The findings of this study suggest the beneficial effect of these plants when introduced in artificial fish feed at 5%.
Keywords: Histopathology, *Aeromonas hydrophila*, *Leucaena leucocephala*, *Ficus benghalensis*, Artificial feed, *Channa punctata*.

INTRODUCTION

Increase occurrence of fish diseases has affected the fish production worldwide. Many chemicals, virus, bacteria and fungus etc. affect fish in many different ways which harm fish organs including organs related to immune system. Many drugs, antibiotics vaccines get accumulated in fish and deteriorate food quality leading to consumer's refusal. Bacterial infections have resulted in disease outbreaks and caused heavy loss to aquaculture throughout the world (1).

Channa punctata, Indian spotted snakehead, is widely distributed in India and common in Indian fish market. Its economic importance has resulted researchers and fish farmers to become more conscious for their health. Different bacteria including *Vibrios sp.*, *Aeromonas* sp., *Pseudomonas* sp. etc. causes severe damage to the fish like kidney, liver, spleen, muscles etc. (2), (3). Infection among fish is very common due to same habitat and the favorable conditions for bacterial growth. Many chemical and biological treatments like antibiotic,

chemotherapeutics, antibodies or vaccines are used to prevent fish from various infections (4), (5). In India, due to lack of awareness regarding fish disease and high expenses involved to undertake preventive measures, farmers meet with inferior fish productivity.

Plants are the rich source of compounds mainly secondary metabolites exhibiting activities like anti-bacterial, anti-fungal, anti-oxidant, anti-inflammatory etc. (6). Plants are rich in nutrition, which function as defensive metabolites for them as well as for other animals including humans. When they are incorporated in the animal feed, they are known to plays a key role in defensive mechanisms. Many studies have been accomplished on use of plant or plant extract in animal feed as a prophylaxis measure as well as for nutritional supplement (7). Use of such plants in feed is important because their use in feed does not inflate laborious expenses and they show beneficial effect on fish health.

Histopathology is widely used for the study of tissue damage due to different chemicals or biological infectious agent as a biomarker worldwide (8). In fish, this parameter has been used since long time to monitor changes in tissue, damage of tissue and pathogen or contaminant concentrations.

This study is an extension of our earlier study (9), which represents the histopathological changes in liver, spleen and kidney tissue in fish after 28 days after introduction of 5% prop root powder of *Ficus benghalensis* and 5% pod seed powder of *Leucaena leucocephala* in artificial fish feed, when successively challenged with *Aeromonas hydrophila* (at weekly intervals) in comparison to control groups.

METHODOLOGY

Experimental design

C. punctata (weight 125±20 grams) were obtained from Gazipur fish market, New Delhi, India and acclimatized to laboratory condition (temp:25°C±1°C; light dark regime of 12L:12D) in 25 L tank. Tank water was changed daily in the morning with dechlorinated water maintained at laboratory conditions. After acclimatization healthy fish were selected (based on their skin texture and activity) for experiment and fed twice a day (morning & evening) at 2.5% body weight with control and experimental feeds accordingly (9). Fish were divided into four groups (I-IV). Group I (Negative control, fish injected with saline and fed on non-supplemented control feed), Group II (Positive control, challenged with *A. hydrophila* and fed artificial non-supplemented feed) andGroup III& IV (Experimental groups challenged with *A. hydrophila* and fed artificial feed supplemented with *F. benghalensis* and *L. leucocephala*, respectively).

Fish were injected successively at weekly intervals with *A. hydrophila* (fish pathogenic bacteria) or carrier fluid (negative control) and fish were sacrificed on day 28. A small portion of liver, kidney and spleen from each groups were excised from the animal and fixed in formalin.

Tissue processing and observation of Histopathology

Formalin fixed tissues were washed overnight under tap water to remove formalin. Tissue blocks were prepared in paraffin wax using a standard protocol. This involved each step wise 15 min (i) dehydration of tissue with alcohol (30-100%), (ii) clearing of tissue with xylene (two changes), iii) fixation in xylene: paraffin (1:1, two changes), followed by (iv) paraffin (two changes). Finally, blocks were prepared by trimming the wax, 6 µm thin sections of tissue were cut using microtome, and spread on slide coated with Mayer's albumin. Slides were heated gently, wax from slide was removed using xylene, tissues were rehydrated through up-gradation of alcohol (100-30%) followed by water (each step 3 min). Slides were then stained using haematoxylin. Tissues were again dehydrated by down-gradation through alcohol (30-

100%) and stained using alcohol based eosin stains. Slides were finally mounted in DPX, observed and photographed under light microscope (Olympus CK30, Japan).

RESULTS

Figure (1) represents a histological section showing histopathological changes in fish liver. Presences of fat vacuole (F), irregular shaped nuclei, nuclear and cytoplasmic degradation of hepatocytes (H) were observed in Group II which indicates damaged or infected liver. Non-significant changes were observed in group III & IV fed on supplementary feed. Increased concentration of Kuffer cells (K) were noticed in challenged groups in comparison to negative control (Group I).

Pathological examination of spleen of Group II (Figure 2 II) showed loosely packed red and white pulp (RP & WP) and collapsed melano-macrophages indicated bacterial infection due to *Aeromonas hydrophila*. Increased white: red pulp ratio indicated activation of immune response in fish. MMCs were also observed in fish groups fed on supplemented feed (Figure 2 III & IV) No such irregularities were observed in negative control (Group I, Figure 2 I).

Alterations in kidney are represented in Figure 3. The most significant change was observed in hematopoietic tissue (HT). Occlusion of tubular lumen and cellular degradation of tubular lining was observed in positive control (Group II). Destructive changes were also shown in proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) in Group II with respect to Group I. There was no such change in Group III and IV fish fed on supplementary feed. Increase in number of melano-macrophage centers (MMCs) were detected in group II, II and IV

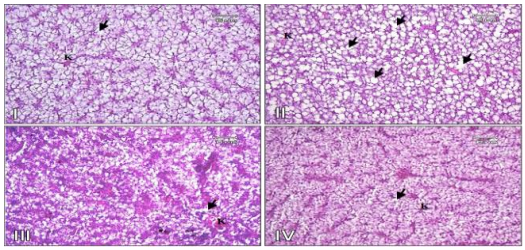

Figure 1

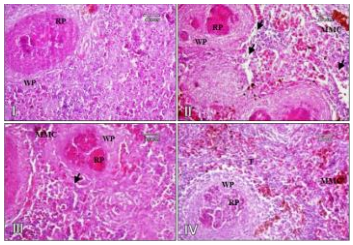

Figure 2

Figure 1: Micrographs depicting the Histopathological/morphological

Figure 2: Micrographs depicting the Histopathological/morphological changes in the spleen of *C. punctata* when challenged with *A. hydrophila.* Group I & II represent negative and positive controls respectively (fed with artificial non-supplemented feed) and Group III & IV represent treatment groups fed with artificial supplementary feeds of *F. benghalensis* and *L. leucocephala* respectively. WP = White pulp, RP = Red pulp, MMC = Melano macrophages center, T = Trabecula, ↙ = degenerative changes. Magnification (400X).

Changes in the liver of *C. punctata* when challenged with *A. hydrophila.* Group I & II represent negative and positive controls respectively (fed with artificial non-supplemented feed) and Group III & IV represent treatment groups fed with artificial supplementary feeds of *F. benghalensis* and *L. leucocephala* respectively. K= kuffer cells, ↙ = fat vacuoles (degradation). Magnification (400X).

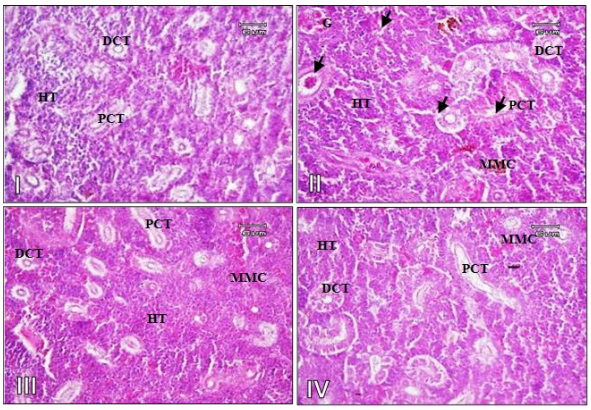

Figure 3: Micrographs depicting the Histopathological / morphological changes in the kidney of *C. punctata* when challenged with *A. hydrophila.* Group I & II represent negative and positive controls respectively (fed with artificial non-supplemented feed) and Group III & IV represent treatment groups fed with artificial supplementary feeds of *F. benghalensis* and *L. leucocephala* respectively. MMC = Melano macrophages center, DCT = Distal Convoluted tubules, PCT = Proximal Convoluted tubules, HT = Hematopoietic tissue, ↙ = destructive changes. Magnification (400X).

DISCUSSION

The histopathological examination showed that the changes occur in most targeting organs of fish like liver, spleen and kidney due to bacterial infection when challenged with *A. hydrophila* (10)-(12). Morphologically pale yellow colored liver reflected fatty changes in liver of group II fish fed on control feed, signifying early to late fibrosis on account of infection by *A. hydrophila*. Such destructive changes in liver lead to dysfunction and damage of hepatic metabolism (8). Large number of fat vacuoles in hepatocyte during microscopic examination represents less glycogen in hepatocyte and indicates injured liver tissue (13), (14). This can be correlated to the increase in the level of hepatic enzymes like SGOT and SGPT. However such changes were not substantial in the liver of fish fed with plant supplementary feeds (Figure 1 III & IV). In comparison to the negative control (Group I, Figure 1 I) there was an increase in Kupffer cells (phagocytic cells) in liver, in group III & IV fed on supplemented feeds (Figure 1III &IV). However their numbers were relatively less in positive control (Group II, Figure 1 II).

Spleen represents lymph node like organ with an important primary lympho-poetic and secondary hematopoiesis function in most of the teleost fish (15). The infected fish spleen showed muddy appearance with loose connective tissue, which indicated that the spleen was degraded in the positive control (Group II). On the other hand the spleen in negative control (Group I) and fish fed with supplementary feeds (Group III & IV) looked healthy with brownish reddish appearance. The stained sections showed presence of loose, collapsed interlobular matrix, and higher number of melano-macrophages. This indicated spleen damage in positive control (Group II,
Figure 2 II) however; there was no significant change in other groups (Figure 2 I, III IV). The macrophage collapse (melano-macrophages), aggregate and increase in number during infection to phagocytize the invading pathogens, which act as an indicator of biotic (bacterial/viral) & abiotic (pollutants/water contaminants) infections (12), (16).

Deivasigamani, 2008 reported head kidney to be the major antibody producer against sheep RBC and specified its capacity to endeavor the immune response (17). But *A. hydrophila* infection cause damage to fish kidney (11), (12). In *C. punctata*, degenerative changes were observed mainly in tubular inner lining of anterior/head kidney and hematopoietic tissue. There

was increase in tissue macrophages i.e. melano macrophage center (MMC), in the head kidney of fish fed on plant supplemented feeds (Group III & IV) in comparison to the positive control (Figure 3). The numbers of MMCs present in negative control group were very less (Figure 3 I). This signifies that besides spleen, head kidney also has important defensive function in fish and it helps in antigen clearance by increasing the number of MMCs (they kill and digest the bacterial cells).

The number of melano-macrophages in groups challenged with *A. hydrophila* increased in comparison to negative control (Group I). Such change indicates the activation of non-specific immune response against disease and stress (18).

But the number of MMC was comparatively higher in group fed on plant supplementary feeds (Figure 3).Hence, this study suggests that these plants can be introduced in feed of cultured fish to prevent bacterial infection and keep fish healthier. Study by Verma et al., 2015 also suggests that *Ficus benghalensis* aerial root powder and *Leucaena leucocephala* pod seed powder at 5% in fish feed shows beneficial effect on fish health and reduced the overall stress of fish due to bacterial infection.

CONCLUSIONS

It is important for the fish farmers to adopt a prophylaxis/ treatment method which is cheap and effective against fish pathogens. Through histological examinations it has been observed that tissues of challenged fish pathogenic bacteria and fed with supplementary feeds suffered less degradation. Therefore, based on these histopathological observations and our earlier study (Verma et al., 2015), we recommend feeding of Indian spotted snakehead, *Channa punctata* with feed supplemented with *Ficus benghalensis* or *Leucaena leucocephala* to fight against *A. hydrophila*.

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