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Histopathology of Skeletal Muscles and Liver of 9 Months Old Labeo Rohita (Hamiltin 1822) Fed with Aqua Fix During Aeromoniasis

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ABSTRACT

The impact of phytogenic immunostimulant, Aqua Fix was studied on the histopathological changes in skeletal muscles and liver of *L. rohita* fed with Aqua Fix during aeromoniasis. Three groups (A,B ,C) of nine months old fish were used in this experiment; groups A and B were treated with Aqua Fix for 4 days, on day 5 fish of group B and C were infected with *Aeromonas hydrophila* @ 10^{-6}CFU/fish(fish of group C were fed with normal diet for 4 days).Controls (group D) were untreated and uninfected. Microscopic examination of the skeletal muscle and liver tissues were carried out on selected days of experimentation (day 1 and day 4). The stained sections were observed under microscope and histological changes were noted. Immunostimulated fish (group A) showed compactly arranged long, unbranched muscle fiber bundles compared to other groups (B and C) of fish. Immunostimulated and infected fish (group B) showed derangement of bundles at certain places, lacunae and odema. Infected fish (group C) showed remarkable changes in the tissue sections. Muscle fibers underwent degeneration and broken completely. The fragments of muscle fibers and lesions were clearly visible. Both the muscle fibers and muscle bundles were healthy in controls (Group D). The liver of immunostimulated fish (group A) showed normal architecture as in control fish. Fish treated with Aqua Fix and infection (group B) showed congestion of central vein and infiltration of cells. Infected fish (group C) showed marked dilation of hepatic vein, haemorrhagic spots, pycnosis of hepatacyte nuclei, necrosis of hepatocytes, inflammation and marked vacuolation at some places. The fish (group B) fed with immunostimulant and infection exhibited moderate effect on skeletal muscle and liver suggesting that Aqua Fix supplementation supports recovery during aeromoniasis.

Keywords: Aqua Fix , Aeromoniasis , *Labeo rohita* , Skeletal muscle, Liver

INTRODUCTION

Aeromonas hydrophila causes a wide range of infections like dermal ulceration, fin and tail rot and hemorrhagic septicaemia in fresh water fishes (Catla , rohu, mrigal, catfish and gold fish). Although it is a normal intestinal flora of fish, it can cause severe disease during stress condition. Aeromoniasis led to outbreak in intensive carp farms (Mohanty et al.2008).Many immunomodulatory substances are found to be effective in rendering protection to aeromoniasis (Sahoo and Sakai 2010).Herbal supplementation

diets exhibited protective immunity in *Cyprinus corpio* (Sunitha et al; 2017) and gold fish (Tangamani et al;2015, Raman 2017). Mixed herbal extracts supplementation diets were found to be triggering the innate immune system of gold fish against *Aeromonas hydrophila* (Harikrishnan et al;2010); the infected fish fed with herbal supplementation diets showed significantly enhanced WBC, haematocrit, protein and glucose level when compared with infected and controlled fish thought the experimental period .The dietary supplement of garlic enhanced superoxide anion production, lysozyme, serum protein and albumin in *Labeo rohita* fingerlings challenged with *A. hydrophilla* (Sahu et al;2017). About 15 plant extracts were found to be effective in conferring the antibacterial activity against *A. hydrophila* in fish (Hanifa and Kavitha 2012). A significant correlation was found in the control of aeromoniasis in cat fish treated with medicinal plants; untreated fish exhibited severe infected lesions leading to death (Thiyagarajan et al; 2014). Dietary incorporation of ethanolic tea leaf extracts enhanced survival of rohu against *A. hydrophila* infection (Sourabh et al;2022).Enhanced protective immune response was observed in fish fed with herbal supplements when exposed to pathogenic microbes (Ukwe and Gabriel, 2019; Ukwe and Abu, 2021; Ukwe and Deekae,2022, 2024). *L. rohita* exposed to Aqua Fix and *A. hydrophila* showed protective immune response in enhancing biomolecules of vital organs (Ankamma and Viveka Vardhani; 2022; 2025; Muthulakshmi and Neelanaryanan; 2025). Immunoprotective effect of microbial levan with regard to enhanced level of haemoglobin content, total WBC and RBC count, serum lysozyme activity, respiratory burst activity, normal hepatocytes and renal tubules was found in *L. rohita* challenged with *A.hydrophila;* degenerated hepatocytes, oedema and leucocytic infiltration in parenchymatous tissues in liver was reported in infected groups (Gupta et al;2008). Manoj et al.,(2010) reported severe proliferation of epithelial cells in gills, conjection of hepatic sinuses, increased number of vacuoles, cloudy swelling in swollen hepatocytes and necrosis of *L. rohita* infected with *A.hydrophila*. Pal et al.,(2015) monitored the ill effects of *A.hydrophila* in *L.roita* with regard to apoptotic death of hepatocytes in liver of *L.rohita*. Fresh water fish (*Clarius batracus*) exposed to sago effluents showed various degree of muscle deterioration (Ramesh and Nagarajan., 2013). Fingerlings of *L. rohita* infected with *Vibrio cholera* showed marked difference in the structure of gill, liver and kidney compared with those fed with *Lactobacillus rhamnosus* and challenged with *V.cholera* (Gobinath and Ramanabai., 2014). Impact of aeromoniasis during treatment with Aqua Fix was found to be marked changes in the architecture of skeletal muscles and liver of *L.rohita* (Ankamma and Viveka Vardhani., 2025). Hence a new vista has been opened to study the pathological changes in muscle and liver of 9 months old *L.rohita* during immunomodulation and aeromoniasis.

MATERIALS AND METHODS

L. rohita, of approximately 9 months (47-50 g) old were collected from local fish farm, Kuchipudi (V), Amrutaluru (M), Guntur (D), Andhra Pradesh, India. Fish were kept in four plastic tubs in laboratory and acclimatized for 7 days. Water quality parameters were regularly monitored and maintained optimally. Fish were fed with commercially available pellet food. Two-third of the water was renewed every day using de-chlorinated ground water to maintain good water quality. Three experimental groups of nine month old *L. rohita* (47-50 g) were selected for the experimentation. Groups, A (treated with Aqua Fix), B (treated with Aqua Fix and infected with *A. hydrophila*), C (untreated with Aqua Fix but infected with *A. hydrophila*) and one control group, D (untreated and uninfected) of fish (10 in each group) were maintained in optimum conditions. Fish of group A and B were fed with a diet supplemented with Aqua Fix (@50mg/100 g of feed) for 4 days; on day 5 fish of group B and C were

infected intraperitonially with *A. hydrophila* @ 10^{-6} CFU/fish. Fish of groups C and D were fed with normal diet (without Aqua Fix) during the entire experimental period.

Bacterial culture

The bacterial strain used in this study, *Aeromonas hydrophila* was collected from State Institute of Fisheries Technology (SIFT), Kakinada, (A.P.), India. This parent culture was subsequently revived by nutrient broth and transferred the culture to a nutrient agar medium. Culture and preparation of isolated bacterial colonies were made under aseptic conditions following the method of Pelczar (1993).

Histopathology

Histological examination of the skeletal muscle and liver tissues were carried out on selected days of experimentation (day 1 and day 4 of experiment). Isolated muscle and liver tissues of control and experimental fish were gently rinsed with normal physiological saline (0.9% NaCl) to remove blood and debris adhering to the tissues and were fixed in 10% formalin. After dehydrating to a graded series of alcohols, the tissues were cleared in absolute methyl alcohol and xylene, and embedded in paraffin wax. Sections were cut at 5 µ thickness and stained with haematoxylin and eosin. After dehydration and clearing, sections were mounted with DPX. The stained sections were observed under microscope and histological changes were noted.

RESULTS

The tissue sections of muscles and liver of 9 months old *L. rohita* displayed marked histological / pathological changes during bacterial infection (groups B– treated with Aqua Fix + infected; C– infected). The fish which were fed with Aqua Fix (group A) and control (group D) showed normal architecture in the muscles and liver throughout the experiments.

Skeletal Muscles:

Microscopic examination:

On day 1 of experiment: Group D(control, untreated with Aqua Fix and infection) (Fig.1, D1): The transverse sections of normal skeletal muscles exhibited long, unbranched muscle fibers (myofibrils) arranged in bundles. The muscle fibers are connected with each other by means of connective tissue. Each muscle fiber showed clear sarcolemma.

Groups A (treated with Aqua Fix), B (treated with Aqua Fix and infected with *A. hydrophila*) and C (infected with *A. hydrophila*) (Fig.1, A1, B1, C1):

Fish treated with Aqua Fix (group A) exhibited long and unbranched muscle fibers in bundles. They were held together and each muscle fiber was encircled by sarcolemma (Fig.1, A1). In comparison with controls (group D) and other experimental fish (groups B, C), muscle fibers appeared healthy in the immunostimulated group (A) of fish. In the immunostimulated and infected group (B) of fish, muscle fibers were slightly disturbed. Lacuna appeared between muscle fibers at some places and attached closely at other places; hypertrophy and sarcolemma degeneration was observed. Each muscle fiber showed branching. Infected fish (group C) showed derangement of muscle fibers. Haemorrhages were seen in the connective tissue with slight destruction and fragmentation and lost their straight appearance and curved like a snake at various places.

On day 4 of experiment:

Group D (control, untreated with Aqua Fix and infection) (Fig.1, D4):

Skeletal muscles showed long, unbranched and intact muscle fibers arranged in bundles. Sarcolemma and nuclei were clearly visible.

Group A (treated with Aqua Fix), B (treated with Aqua Fix and infected with *A. hydrophila*) and C (infected with *A. hydrophila*) (Fig.1, A4, B4, C4)

Immunostimulated fish (group A) showed compactly arranged long, unbranched muscle fiber bundles. The striated muscle fibers were enclosed in specialized cell membrane, plasmalemma. Each muscle fiber (large cell) is multinucleated. Each myofibril is composed of two short myofilamentes; the arrangement of these myofilaments giving the appearance of transverse banding. Immunostimulated and infected fish (group B) showed derangement of bundles at certain places. Lacunae were visible at certain points because of vacuolar degeneration and odema. Infected fish (group C) showed remarkable changes in the tissue sections. Muscle fibers underwent degeneration and broken completely. The fragments of muscle fibers and lesions were clearly visible.

Liver:

On day 1 of experiment: Group D (control, untreated with Aqua Fix and infection) (Fig.2, D1):

Histology of normal liver showed large, polygonal hepatocytes with almost centrally placed nuclei. Compact hepatocytes showed prominent central vein, sinusoids and well developed hepatic chords.

Groups A (treated with Aqua Fix), B (treated with Aqua Fix and infected with *A. hydrophila*) and C (infected with *A. hydrophila)* (Fig.2, A1, B1, C1)

The liver of immunostimulated fish (group A) showed normal architecture as in control fish. Hepatic chords, sinusoids, large and polygonal hepatocytes with centrally placed nuclei were clearly visible. In case of immunostimulated and infected fish (group B), multiple fibromas and focal necrosis of hepatocytes were observed. Also, histological sections showed dilation of sinusoids, inflammation, deep vacuolization and pycnosis of nuclei in the hepatocytes. The liver of infected fish (group C) showed odema, focal necrosis, inflammation, loss of parenchymatous tissue and infiltration of cells. Also, liver tissue showed formation of vacuoles and granulation, and filled with necrotic debris.

On day 4 of experiment:

Group D (control, untreated with Aqua Fix and infection) (Fig.2, D4):

The histological architecture of liver was similar to day 1 on day 4 of experiment and liver showed normal architecture.

Groups A (treated with Aqua Fix), B (treated with Aqua Fix and infected with *A. hydrophila*) and C (infected with *A. hydrophila)* (Fig.2, A4, B4, C4):

The histopathological changes observed in immunostimulated fish (group A) were similar to those observed on day 1 of experiment. Large hepatic cells were polygonal in shape; well-developed hepatic chords, sinusoids and parenchyma were found. Fish treated with Aqua Fix and infection (group B) showed congestion of central vein and infiltration of cells. Sinusoids were heavily dilated. Hepatocytes underwent necrosis. The parenchymatous pulp was occupied by cell debris and granulomatous inflammation. Pycnosis of hepatocyte nuclei was also observed. Infected fish (group C) showed marked dilation of hepatic vein, haemorrhagic spots, pycnosis of hepatacyte nuclei, necrosis of hepatocytes, inflammation and marked vacuolation at some places.

DISCUSSION

The histopathological changes observed on day 1 and 4 of experiment in skeletal muscles of infected fish displayed derangement of muscle fibers, lacunae, lesions and necrosis. Appearance of lacunae, odema and vacuolar degeneration were found in the channel catfish infected with *A. hydrophila* (Ventura and Grizzle, 1988) and muscular necrosis in Tilapia (Azad *et al*., 2001) during *A. hydrophila*

infection. Bach *et al.,* (1978), Candan (1990) and Moore *et al.,* (1998) recorded muscular necrosis, odema, inflammation and infiltration of cells in channel catfish – *I. punctatus*, in rainbow trout – *S. giardineri* and in *C. carpio* during *A. hydrophila* and viraemia infections. The present findings on histopathological changes in skeletal muscles confirm that of Harikrishnan *et al.,* (2009) who found fragmentation of muscle fibers with necrosis and sarcoplasmic debris in goldfish, *C. auratus* infected with *A. hydrophila* and Manoj *et al*., (2010) who also found local haemorrhages, dermal odema and muscular degeneration in *L. rohita* infected with *A. hydrophila* by immersion challenge. Fish supplemented with Aqua Fix and infected showed less severe histopathological changes in skeletal muscles compared to other infected groups indicating the protective effect of Aqua Fix. These results are in agreement with that of Jeney and Anderson (1993), Siwicki *et al*., (1994), Selvaraj *et al.,* (2005), Rairakhwada *et al*., (2006) and Gupta *et al.,* (2008) who stated that supplementation of immunostimulants improve the health status of fish. Diet supplemented with Aqua Fix might have increased resistance to fight with bacterial pathogens or to neutralize the toxic effect of pathogens.

The fish which received immunostimulant and infection showed moderate effect on liver suggesting that Aqua Fix supplementation ameliorate the ill effects of *A. hydrophila*. Various authors (Selvaraj *et al*., 2005; Rairakhwada *et al*., 2006; Gupta *et al*., 2008) documented the positive influence of immunostimulants in freshwater fish which in turn retains the normal histology to certain extent compared to other groups.

Histopathological changes in liver of 9 months old *A. hydrophila* infected fish characterized by necrosis of hepatocytes, vacuolization and pycnosis of nuclei, haemorrhages, dilation of hepatic veins, derangement of sinusoids and inflammation. Similar type of histological changes in tissues of liver, kidney and intestine were noticed by Huisinga *et al*., (1979) in *M. salmoides*, Angka (1990) in *C. batrachus*, Candon in *S. giardneri* (1990) and Miyazaki *et al*., (2001) in *C. carpio* during *A. hydrophila* infection. Harikrishnan *et al.,* (2009) also found granulomatous inflammation and necrosis of hepatocytes in *C. auratus* infected with *A. hydrophila*. Manoj *et al*., (2010) recorded haemorrhages, swollen hepatocytes, inflammation and derangement of hepatocytes in *L. rohita* infected with *A. hydrophila*. Liver tissue showed swollen hepatocytes, dilation of arteries and veins and haemorrhagic spots in carrageenan infected *L. rohita* (Vikash Kumar *et al*., 2014).

CONCLUSION

The results shown in the present study indicated that the immune system in 9 months age group of fish was modulated by dietary supplementation with Aqua Fix and responded to resist *A. hydrophila* infection. *L. rohita* fed with Aqua Fix had a protective effect on skeletal muscles with marked structural integrity and restoration of liver architecture when compared to controls and other groups. These findings suggest that inclusion of Aqua Fix as feed additive modulates immunity in *L.rohita* against aeromoniasis and improves health status and disease resistance. However, further studies are needed to use Aqua Fix as immunostimulant in other fishes and against other microbial pathogens as herbal immunostimulants are cost effective and offer promising eco-friendly approach to strengthen sustainability and productivity in aquaculture.

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Figure.1.Showing the section of skeletal muscles of *L. rohita* (9 months old) from group D (control, untreated with Aqua Fix and uninfected), group A (Aqua Fix treated @ 50mg/100g of feed), group B (treated with Aqua Fix @ 50mg/100g of feed+ infected with *A.hydrophila* @ 10^{-6} CFU/fish), and group **C (infected with *A.hydrophila* @ 10^{-6}CFU/fish) on day 1 and 4 of experimental period. MF – Muscle fiber; MB – Muscle bundle; DMF – Derangement of muscle fibers; DSL – Degeneration of sarcolemma; H – Haemorrhages; MFD – Muscle fiber destruction; FMF – Fragmentation of muscle fibers; - LE Lesion.**

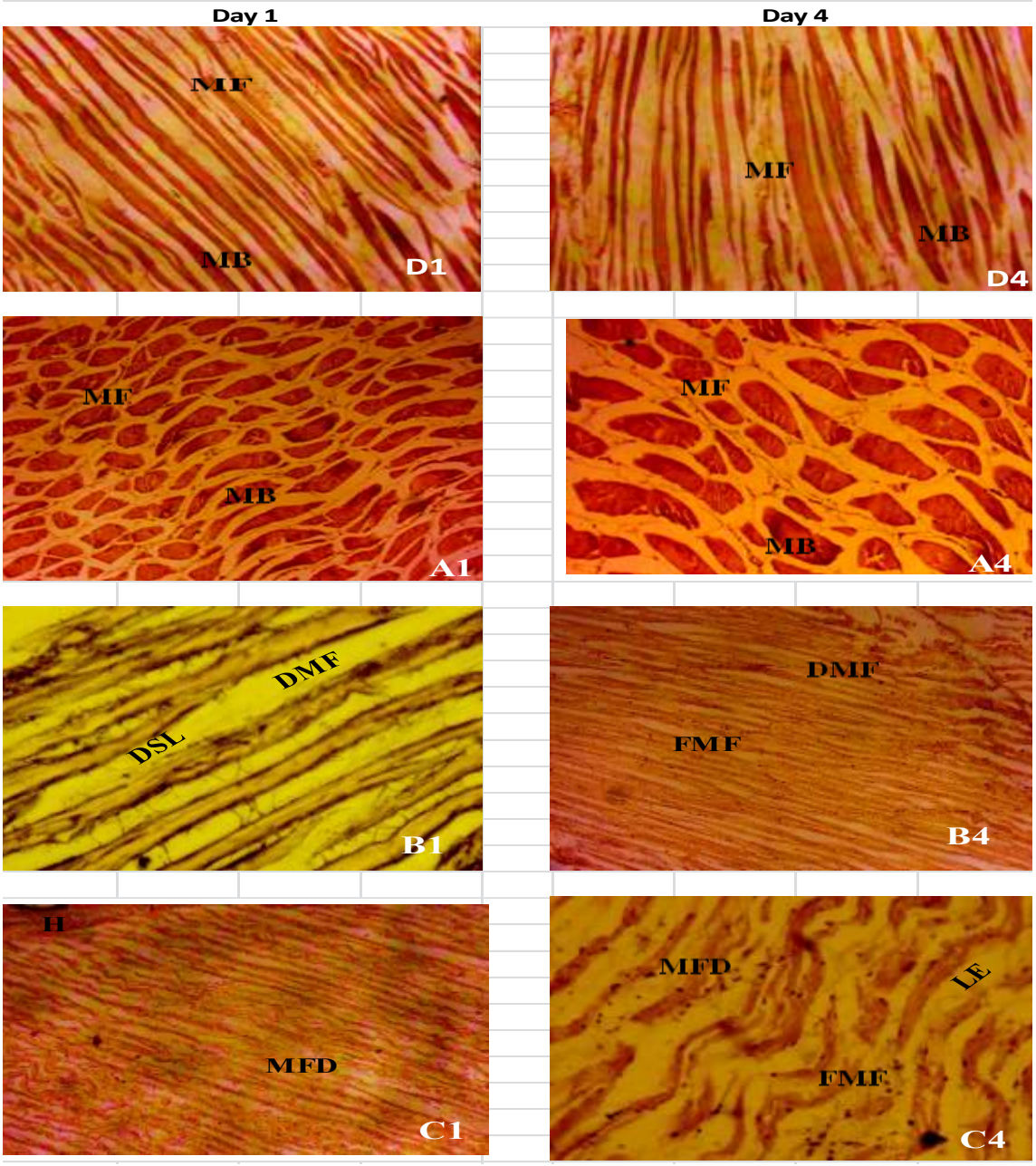
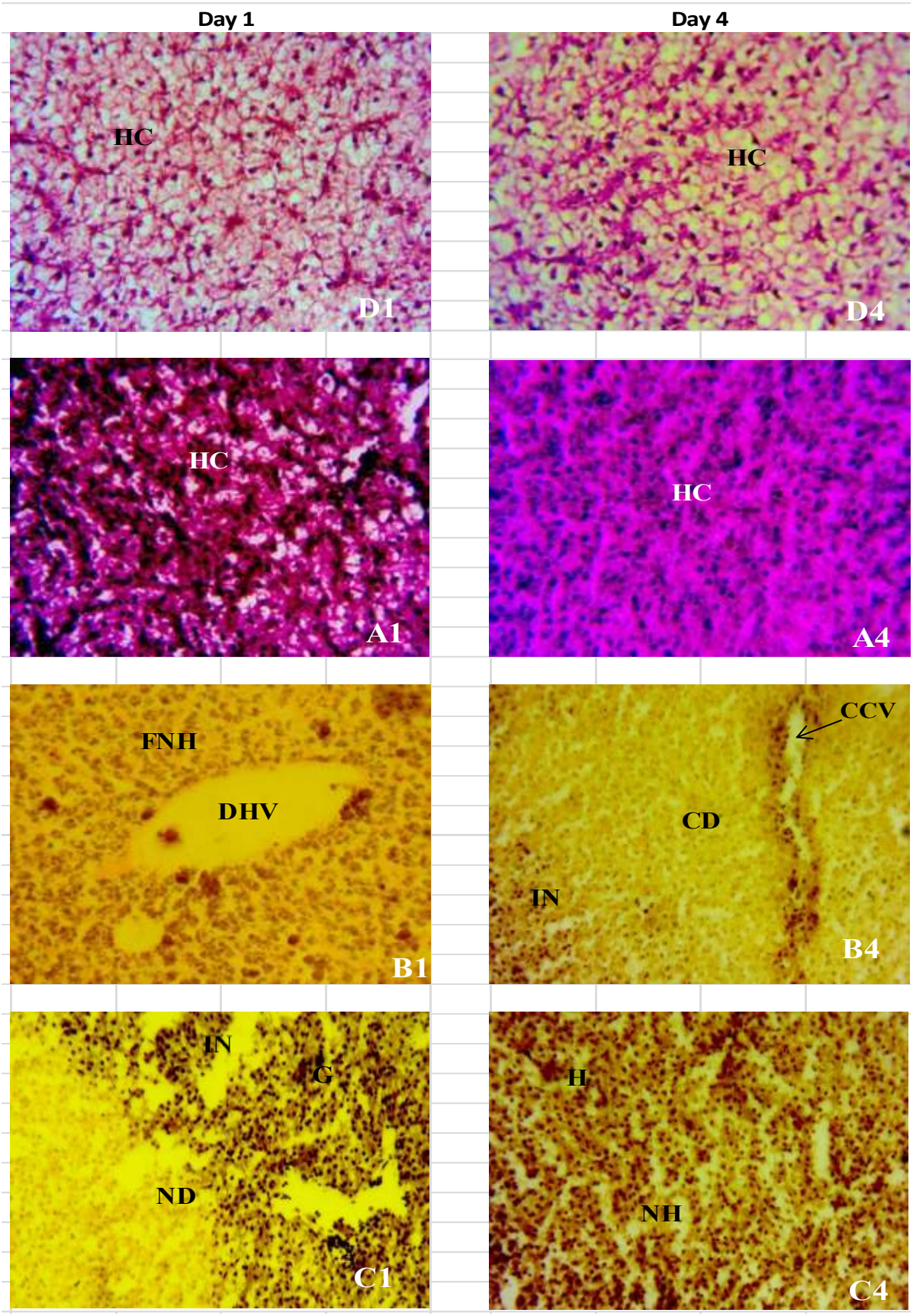


Figure.2.Showing the section of liver of *L. rohita* (9 months old) from group D (control, untreated with
Aqua Fix and uninfected), group A (Aqua Fix treated @ 50mg/100g of feed), groupB (treated with Aqua Fix @ 50mg/100g of feed+ infected with *A.hydrophila* @ 10^{-6} CFU/fish), and group C (infected with *A.hydrophila* @ 10^{-6}CFU/fish) on day 1 and 4 of experimental period. HC – **Hepatocytes;** FNH – **Focal necrosis of hepatocytes;** DHV – **Dilation of hepatic vein;** IN – **Infiltration;** G – **Granulation;** ND – **Necrotic debris;** CCV – **Congestion of central vein;** CD – **Cell debris;** H – **Haemorrhages;** NH – **Necrosis of hepatocytes**


Day 1
Day 4
HC
D1
HC
D4
HC
A1
HC
A4
FNH
DHV
B1
CCV
CD
IN
B4
IN
G
ND
C1
H
NH
C4