CHAPTER VIII HISTOPATHOLOGY

8.1 INTRODUCTION

Fish gastrointestinal tract is one of the major infection tracts. It presents a favourable medium for bacteria multiplication (Robertson *et al.*, 2000; Ringo *et al.*, 2012). Fishes come into contact with multiple harmful microbes and toxins in the aquatic environment which cause multiple diseases and result in the death of the organisms. The tissue lesions and apoptosis arise from infections, diseases, parasites and bioaccumulation of toxins stimulate necrotic alterations in the fish with an inflammatory defensive reaction (Roganovic Zafirava and Jordanova, 1998). Bacterial infection in the fishes causes metabolic disturbances that induce a cell and organ level damage.

Histopathology is a suitable tool for monitoring and diagnosing of health, where the changes at the cells and tissues due to the pathogen are interpreted to arrive at diagnosis (Ambipillai *et al.*, 2003). A number of diseases in fishes have been reported and the vital causative agents are pathogenic viruses, bacteria and parasitic organisms. Fish can easily stressed by improper handling which in turn causes diseases.

Antibiotics inhibit or kill beneficial microbiota in the gastrointestinal (GI) ecosystem. The antibiotic residues accumulated in fish products are found to be harmful for human consumption (WHO, 2006). For the above reason European Union has ratified a ban since 2006 for the use of all sub-therapeutic antibiotics as growth-promoting agents in animal production. Richard and Antony (1983) have reported some cases of drug overdose leading to fish death and other detrimental side effects. The screening of plant extracts and natural products for antimicrobial activity has shown
that higher plants represent potential sources of new anti infective agents (Press, 1996). Plant derived phyto medicines have great promise in the treatment of infectious diseases. Phytochemicals could be an alternative to the chemotherapeutic molecules and are found to be safe to use in aquaculture (Punitha et al., 2008). The development of the piscine gonads has been described in terms of stages and maturity (Treasurer, 1990; Ha and Kinzie, 1996). Gonadal maturity depends upon the quality and quantity of food. Gonad weight, gonado somatic index, sexual maturation and spawning time are controlled by nutritious artificial diet (Singh and Singh, 1979). According to Watanabe (1990), nutrition has profound effect upon gonadal growth. Gonadal weight is related to the maturity and spawning process which depends on multiple factors such as sex, maturity stage, individual size, season or reproductive investment. The morphological changes in the ovary coincide with the histological studies of gonads (Palmer et al., 1995; Ha and Kinzie, 1996).

8.2 MATERIALS AND METHODS

In the present study maximum growth rate and survival of the chosen fish *Cyprinus carpio* was found in Ar 1.5%, Ws 1.5% and Mp 2.0% formulated feed. Hence for histopathological studies control and herbal diet fed fishes (Ar 1.5%, Ws 1.5% and Mp 2.0%) were selected to study the influence of herbal diets on the histological changes in chosen tissues. They were subjected to bacterial infection. After 21 days of bacterial exposure, the test fish were sacrificed to get the necessary tissues for histological studies. The organs chosen for the present study were gill, liver, muscle and intestine. The histomorphological study of ovary in herbal diet fed fishes in Ar 1.5%, Ws 1.5% and Mp 2.0% were also selected for their proven efficiency in the
gonadal development. Other details were given in general Materials and Methods (chapter 3).

8.3 RESULTS

GILLS -NORMAL HISTOLOGY

The gill rachis from which the filaments of gills arise is supported by a skeleton comprising a core cartilage which is enveloped by connective tissue sheath. The soft parts of gills are composed of several layers of cells, the outermost being the thin epithelium. The gills in the control fish was characterized by the presence of primary and secondary lamellae. The primary lamellae were thickened. The secondary lamellae were straight and evenly distributed. (Plate 5a and 5b). The respiratory surface of each gill filament, which is finger shaped, is formed by the outermost epithelium. These epithelial cells are mostly isodiametric and in places they are cubical or even columnar. Scattered in the epithelial layer are several mucus cells which secrete mucus consist of neutral mucopolisaccharides. The mucus cells are goblet shaped and their vacuole like cavities are filled by mucus in the form of large granules. The epithelial cells have prominent nuclei and nucleoli. In refined sections of the gill, the arrangement of the capillaries forms a meshwork in the gill ensuring through vascularisation of the gill. (Plate 5a and 5b).

Histopathological changes in Gills: Control fishes (Cyprinus carpio) when infected with A. hydrophila showed the epithelial necrosis with intracellular oedema (Plate 5c). The damaged portions caused by necrosis of gill epithelium were accumulated. Another section of a gill shows complete erosion of the respiratory surface showing only the degenerated part of the cartilage skeleton of gill filament. The gill filaments appear hypertrophied because of necrosis (Plate 5d, 5e and 5f). The gill photomicrograph
PLATE 5

Histological observations of gill of *C. carpio*

Plate 5a Control gill - (Haematoxylin and Eosin X 400)

Plate 5b Control gill - (Haematoxylin and Eosin X 1000)

Plate 5c, 5d, 5e and 5f: Gill of Control fishes challenged with *A. hydrophila*  
(Haematoxylin and Eosin X 400)

PL  –  Primary Lamella
SL  –  Secondary Lamella
NC  –  Epithelial Necrosis
EM  –  Intracellular edema
ECS –  Epithelio capillary Separation
EH  –  Epithelial hyperplasia
DSQ –  Epithelial Necrosis with desquamation
Plate 5. Histological observations of gill of *C. carpio*
PLATE 5.1

Histological observations of gill of herbal diet fed fishes challenged with

*A. hydrophila*

Plate a, b, c, d, e and f

PL  –  Primary Lamellae

SL  –  Secondary Lamellae

NC  –  Epithelial Necrosis

ECS –  Epitheliocapillary Separation
Plate 5.1. Histological observations of gill in herbal diet fed fishes challenged with *A. hydrophila*
showed distortion of the lamella with overlapping of the primary and secondary lamella. They exhibited extensive gill damage with epitheliocapillary separation. Mild congestion of blood vessels were seen in the primary lamellae, and marked epithelial hyperplasia of the branchial arch was evident. Considerable mucous and granulated eosinophilic cells were witnessed in their cytoplasm. Extensive vacuolization were observed with prominent disruption of epithelium. The lesions were associated with desquamation of the epithelium. The histology of the gill tissues of herbal diet fed fishes showed mild to moderate congestion of the primary lamellae and exhibited no vacuolization. The primary lamellae were thickened. The secondary lamellae were straight and evenly distributed. The gill lamellae are not fused. (Plate 5.1. a, b, c, d, e and f). Mild hypertrophy of lamellar epithelium and necrotic changes were visible.

**LIVER-NORMAL HISTOLOGY**

The hepatocytes, which are the parenchyma cells of the liver, are grouped into lobules in the liver. These lobules radiate around a central view. The rows of hepatocytes form laminae or plates. Large lacunae separate the liver cell-plates. The veins of the liver open into large venous sinusoids. The hepatocytes are polygonal and isodiametric. These cells have prominent nuclei with dense chromatin. The nucleoli are very conspicuous in the hepatocytes and are one or two in number. Some vacuoles are small and irregular. The vascular regions of the sections of the liver show oval nucleated RBC and other blood cells. There is moderate storage of glycogen in the form of small and large granules in the cytoplasm of the hepatocytes. Blood sinusoids were observed (Plate 6b).
PLATE 6

Histological observations of liver of *C. carpio*

Plate 6a Control liver - (Haematoxylin and Eosin X 400)
Plate 6b Control liver - (Haematoxylin and Eosin X 1000)
Plate 6c, 6d, 6e and 6f- liver of Control fishes challenged with *A. hydrophila* –
(Haematoxylin and Eosin X 1000)

NLP – Normal liver parenchyma
SS – Dilation of Sinusoids
HC – Hepatocytes
ND – Necrotic debris
VO – Hyper vacuolation
DS – Dilation of sinusoids
MP – Macrophages
NH – Necrotic hepatitis
DH – Deformed hepatocytes
LC – Lymphocytes
Plate 6. Histological observations of liver of *C. carpio*
PLATE 6.1

Histological observations of liver of herbal diet fed fishes challenged with *A. hydrophila*

Plate 6.1 a and b – (Haematoxylin and Eosin X 400)

Plate 6.1 c, d, e and f – (Haematoxylin and Eosin X 1000)

NLP  –  Normal liver parenchyma

SS   –  Dilation of Sinusoids

HC   –  Hepatocytes

VO   –  Vacuolation

MP   –  Macrophages

GR   –  Granulomatous reaction
Plate 6.1. Histological observations of liver in herbal diet fed fishes challenged with *A. hydrophila*
Histopathological changes in liver

The normal architecture of liver tissue was markedly disrupted in control fishes infected with *A.hydrophila*. The liver developed vacuoles. Highly vacuolated irregular liver cells with necrotic nuclei are seen. Due to the accumulation of more vacuoles, the liver has lost its structure. The degeneration is seen to set in almost all the cells. Cells have become more debris and they make clusters of disorganized cells and appear as small and large faint dots among large vacuoles. Macrophage granulomas were observed. The liver shows the lysis of several hepatocytes and large vacuoles known as necrotic debris (Plate 6 c, 6 d, 6 e and 6 f). Hypertrophy of hepatocytes, lymphocytes and clumping was also evident in many places. Free pre-granulomatous tissue, acute necrotic hepatitis i.e. widespread damage to the micro vascular system resulting in subcutaneous bleeding and blackening of the skin, and dilation of sinusoids were also observed with indistinct cell boundaries in many places.

The histology of the liver tissues of herbal diet fed fishes exhibited normal liver parenchyma cells. The hepatocytes are polygonal and isodiametric. Minimum granulocytes were noticed. No hyper vacuolization were seen. The dilation of sinusoids was limited. Macrophage granulomas were observed (Plate6.1. a, b, c, d, e and f).

**MUSCLE-NORMAL HISTOLOGY**

The photomicrograph of the muscle (Plate7a and7b) depicted the presence of a group of tissues formed from somites termed as myotomes with equally spaced muscle bundles. Normally the muscle comprises of two compactly arranged distinct layers with an outer red muscle layer and inner white muscle layer.
PLATE 7

Histological observations of muscle of *C. carpio*

Plate 7a and b Control muscle - (Haematoxylin and Eosin X 400)

Plate 7c, d, e and f Muscle of Control fishes challenged with *A. hydrophila* –
(Haematoxylin and Eosin X 1000)

- MB: Muscle bundle
- SMB: Short muscle bundle
- G: Wider gaps between muscle bundle
- SDMB: Severe damage of muscle bundle
- NMB: Necrosis of muscle bundle
- TMB: Thickening of muscle bundle
- SIO: Severe intra muscular edema
Plate 7. Histological observations of muscle of *C. carpio*
PLATE 7.1

Histological observations of muscle of herbal diet fed fishes challenged with *A. hydrophila*

Plate 7.1  a, b, c, d, e and f (Haematoxylin and Eosin X 400)

MB -  Muscle bundle

SMB -  Short muscle bundle

TMB -  Thickening of muscle bundle
Plate 7.1. Histological observations of muscle in herbal diet fed fishes challenged with *A. hydrophila*
HISTOPATHOLOGICAL CHANGES IN THE MUSCLE

The histopathology of the muscle tissues of *C. carpio* in the control fishes on exposure to *A. hydrophila* infection, showed marked thickening and separation of muscle bundles, severe damage of muscle bundles, haemolysis, dermal lesions, severe intramuscular oedema with major dystrophic changes, shortening of muscle bundles, and necrosis of muscle bundle were also observed. Infiltration of macrophages, Granulomatous inflammation and fragmentation of muscle bundles were also noticed. There was wider gap between muscle bundles (Plate 7c, d, e and f). The histology of the muscle tissues of herbal diet fed fishes also showed some degenerative changes like, shortening of muscle bundles, thickening of muscle bundles. Mild lesions were noticed. Moderate necrosis was also observed. (Plate 7.1a, b, c, d, e and f).

INTESTINE -NORMAL HISTOLOGY

The intestine is a long tubular structure and its transverse section reveals a very simple histological organization. Normal structure of fish intestinal tract with three intestinal layers such as Serosa, muscularis and muscularis mucosae with highly developed villi. The mucosa consists of one layer of epithelial cells forming the lining of the lumen. The sub mucosa consists of connective tissue. Occasionally, discontinuities occur in the epithelial layer and the phagocytes find these as ideal places for exit or entry. Goblet cells which secrete mucopolysaccharides are seldom visible in the mucosal lining of the control fish (Plate 8 a, 8 b).

HISTOPATHOLOGICAL CHANGES IN THE INTESTINE

The histopathology of the intestine of *C. carpio* in the control fishes on exposure to *A. hydrophila* infection showed that the epithelium of the tips of villi are corroded. In
PLATE 8
Histological observations of intestine of *C. carpio*

Plate 8 a Control intestine – (Haematoxylin and Eosin X 400)
Plate 8 b Control intestine – (Haematoxylin and Eosin X 1000)
Plate 8 c, d, e and f Intestine of control fishes challenged with *A. hydrophila* – (Haematoxylin and Eosin X 1000)

M – mucosa
L – Lumen
SM – Sub mucosa
SR – Serosa
NC – Necrosis
MC – Mucosa
AT – Atrophy of intestinal villi
MN – Mucosal necrosis
NL – Necrosis of lumen
EM – Sub mucosal edema
Plate 8. Histological observations of intestine of *C. carpio*
PLATE 8.1

Histological observations of intestine of herbal diet fed fishes challenged with *A. hydrophila*

Plate 8.1 a - (Haematoxylin and Eosin X 400)
Plate 8.1 b - (Haematoxylin and Eosin X 400)
Plate 8.1 c, d, e and f - (Haematoxylin and Eosin X 1000)

L - Lumen
SM - Sub mucosa
M - Mucosa
SR - Serosa
MS - Muscularis
NE - Necrosis
MC - Mucosal epithelium
AC - Absorptive cell
GC - Goblet cells
Plate 8.1. Histological observations of intestine in herbal diet fed fishes challenged with *A. hydrophila*
some regions the villi have become very wide, conical and swollen. In these areas, the intestine has lost all its structural organization. Sloughing of mucosal epithelium, loss of absorptive and goblet cells and villus atrophy were noticed. Morphological alterations observed at the anterior intestine like epithelium lesion, and detachment of intestinal layers. The fishes exhibited the hyper vacuolations of intestinal mucus membrane, mucosal necrosis and sub mucosal oedema.(Plate 8c, d, e and f).

The histology of the intestine of herbal diet fed fishes exhibited a mild alteration in the intestinal layers, moderate lesions in the intestinal villi. Internally the structures remain intact. Accumulation of goblet and absorptive cells were observed which shows that the herbal diets significantly increased the resistance to *A. hydrophila* infection (Plate 8.1a, b, c, d, e and f). The present study has clearly demonstrated the potency of *M. pruriens* to reduce the intestinal damage caused by *A. hydrophila* (Plate 8.1 e and f).

**HISTOMORPHOLOGICAL STUDY OF OVARY**

(Plate 9a, b), (Plate: 10a, b), (Plate: 11a, b, c). Section of the ovary in the region of the primordial germ cell shows immature germinal tissue consisting of small cells. After the oogonia give rise to the primary oocytes, the crop of oocytes undergo growth. During this stage, they appear polyhedral with flat adhering sites due to close packing. Ultimately, the oocytes become spherical or sub spherical and appear circular or elliptical in sections. The oocytes are enclosed by a thin layer of follicular epithelium. A developing oocyte has an apparently homogenous cytoplasm and a large nucleus, called germinal vesicle (Plate: 10e, 11e). The chromatin material or the chromosomes are diffused in the early stages of development. When the nuclei of the primary oocytes are in the early diplotene stage, numerous small nucelolar extrusions which are
PLATE 9

Histomorphological study of ovary of *C. carpio* fed with *A. racemosus* formulated feed (Haematoxylin and Eosin X 400)

ZP — Zona pellucida
YG — Yolk granules
PVF — Primary Vitellogenic follicle
NU — Nucleus
GV — Germinal Vesicle
LD — Lipid droplets
Plate 9. Histomorphological study of ovary of *C. carpio* fed with *A. racemosus* formulated feed
PLATE 10

Histomorphological study of ovary of *C. carpio* fed with *W. somnifera* formulated feed (Haematoxylin and Eosin X 400)

YG  –  Yolk granules
DO  –  Developing Oocytes
ZP  –  Zona pellucida
LD  –  Lipid droplets
NU  –  Nucleus
PNU – Peri nucleolus Follicles
Plate 10. Histomorphological study of ovary of *C. carpio* fed with *W. somnifera* formulated feed
**PLATE 11**

*Histomorphological study of ovary of C. carpio fed with M. pruriens formulated feed (Haematoxylin and Eosin X 400)*

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO</td>
<td>Developing Oocytes</td>
</tr>
<tr>
<td>BC</td>
<td>Basophilic Cytoplasm</td>
</tr>
<tr>
<td>PNU</td>
<td>Perinucleolus Follicles</td>
</tr>
<tr>
<td>YG</td>
<td>Yolk granules</td>
</tr>
<tr>
<td>ZP</td>
<td>Zona pellucida</td>
</tr>
<tr>
<td>ZR</td>
<td>Zona Radiata</td>
</tr>
<tr>
<td>NU</td>
<td>Nucleus</td>
</tr>
<tr>
<td>MN</td>
<td>Migratory Nucleus</td>
</tr>
</tbody>
</table>
Plate 11. Histomorphological study of ovary of *C. carpio* fed with *M. pruriens* formulated feed
spherical, darkly staining bodies appear within the nucleus and move to the periphery. These are like nucleoli and ultimately they move into the perinucleolar cytoplasm through the nuclear membrane. In the cytoplasm of the oocyte there is usually one large spherical basophil structure called the yolk nucleus (Nath, 1968). Occasionally, there may be more than one yolk nucleus in some oocytes. Vitellogenesis begins in older oocytes and the nuclear extrusions present in the cytoplasm can be distinguished from the yolk granules. Several oocyte stages, ranging from primary forms to vitellogenesis, suggest that the maturation was generally successfully attained. Pre-vitellogenesis oocytes (oogonia to perinuclear stage) and more advanced vitellogenesis (primary vesicle to tertiary yolk vesicle) oocyte stages were all found in the same gonads (Msiska, 2002). The nuclear membrane is lobed throughout its circumference during the stage in which nucleolar extrusions are produced. Later stages of vitellogenesis show formation of yolk granules in the cytoplasm. These granules are of varying sizes and intensely stained by iron-haematoxylin. Histological studies of dissected ovaries indicated that different follicles were present.

**CHROMATIN-NUCLEOLUS FOLLICLES:**

Multiple nucleoli were present in the nucleus that arranged irregular, then, they lied at peri nuclear position. Ooplasm was thin and basophilic. (Plate: 11d).

**Perinucleolus Follicles:**

Numerous large nucleoli were at the periphery of the nucleus. The ooplasm contained the nuclear complex of organelles. (Plate: 9f)
Cortical Alveolus Follicles:

Cortical alveoli appeared at various depths in the ooplasm. Also, there were small lipid droplets around the nucleus. Zona radiata appeared as a thin band (Plate: 11d). Chromatin-nucleolus, peri nucleolus and cortical alveolus follicles are pre-vitellogenic follicles. Primary, secondary, tertiary vitellogenic and mature follicles are vitellogenic follicles.

Primary Vitellogenic Follicles: Yolk spheres lied between the cortical alveoli. (Plate: 9a).

Secondary Vitellogenic Follicles: The yolk spheres were moved toward the center and cortical alveoli and lipid droplets moved periphery (Plate: 10f). The nucleus membrane became irregular. The zona radiata thickness and follicles diameter were reached to maximum in this stage (Plate: 11d). The nucleus gradually displaced toward the animal pole. Later, nuclear membrane was disappeared.

Tertiary Vitellogenic Follicles: The yolk spheres were increased and combined together and totally filled the ooplasm. The nucleoli gradually moved toward the center of the nucleus (Plate: 11f).

8.4 DISCUSSION

As there is scanty literature available on the histopathological changes in fish, exposed to bacterial infection and in particular the response of herbal diet fed fishes challenged with Aeromonas hydrophila, the author of the present investigation could not compare the present results with the related publications. However the comparison was made with the pathological changes obtained in the gill, liver, intestine and muscle
tissues of fishes. These organs always come into direct contact with the environment. These are highly vulnerable to bacteria and pollutants.

**GILL**

The morphology and ultra structure of the gills have been well documented by Laurnet and Dunel (1980). Histopathological changes in diseased animals provide a methodological platform to determine the causes of their mortality (Iwalokun et al., 2001). Histopathological changes in *A. hydrophila* infected catfish, *Clarias batrachus*, and rainbow trout, *Salmo gairdneri* have been documented (Angka, 1990; Candan, 1990). Miyazaki *et al.* (2001) noted that skin lesions exhibited a separated epidermis, expanded scale-sacs, and an edematous dermis accompanied by hemorrhage and necrosis in *Cyprinus carpio* by *A. hydrophila* infection. In the present study also skin lesions and necrosis of gill lamellae appeared which shows heavy intensity of infection in control diet fed fishes. Gills of fish for respiration not only exchange gases, but also serve in absorption (or) elimination of ions by diffusion. These organs should be healthy to carry out their efficient functions. Since the gills must allow diffusion of oxygen and Carbon dioxide they cannot be protected (or) coated with any hard covering (Madasamy, 2001).

Harikrishnan *et al.* (2009) observed that irregular aggregates of macrophages in gill lamellae, gill congestion, and hyperaemia in *A. hydrophila* infected gold fish, *Carassius auratus*. The gill lesions were found to be associated with thrombosis, oedema, haemorrhage, desquamation of the respiratory epithelium, and formation of haemorrhage globe. He also observed that the gills after day 30 of 1% tri-herbal concoction using Neem, Tulsi and Turmeric dip treatment showed the presence of oedematous spaces, proliferation of granular cells, and thickening of the secondary
lamellae, fibrosis, and infiltration of leukocytes. In the present investigation also gill congestion and desquamation of the respiratory epithelium were observed. Thickening of the secondary lamellae were noticed in the herbal diet fed fishes. Oedematous spaces are important in the activation of mucosal immune response in different fishes (Demers and Bayne, 1994; Miyazaki et al., 2001).

LIVER

Liver is a vital organ, which performs more of the function including storage of carbohydrates and detoxification. The liver as the centre of metabolism is also involved in breaking down toxic substances. As a result, hepatic cells are subjected to more damage than cells of other organs (Okwari et al., 2000). In largemouth bass (*Micropterus salmoides*), *A. hydrophila* infection leads to necrosis in the liver, heart and kidney (Huizinga et al., 1979). In the present study the infected liver of control diet fed fishes showed necrosis and dystrophic changes of hepatocytes with hypervacuolization. Fischer et al. (2000) indicated that hepatocyte necrosis represents primary events in microcystin induced hepatotoxicity in the rainbow trout and that apoptotic cell death seems to be of only secondary nature. Focal necroses (mainly perivascular) and dystrophic changes of hepatocytes with vacuolisation and nucleic damage (pyknosis, karyolysis, hyperchromatosis, karyorrhexis) were found in the rainbow trout (*oncorhynchus mykiss*) fishes exposed to the medium concentration of the cyanobacteria (*Microcystis aeruginosa*) extract. Apoptotic cells were also detected in the liver, mainly in perivascular and interstitial liver tissue (Palikova et al., 2004). Interstitial haemorrhage and inflammatory cells were found in dorsal musculature and intestine (Angka, 1990). Hepatocytes were either atrophied or swollen and had a granular appearance (Miyazaki et al., 2001). Rodger et al. (1994) described the changes
in liver which were characterized by confluent necrosis showing cellular degeneration and loss of obvious cell boundaries, the hepatic cells were large and polygonal in shape with almost centrally placed nuclei and blood sinusoids. Multiple fibromas and macrophage granulomas were also observed in the brown trout (*Salmo trutta*) infected by the death of water blooms of *Anabaena flos-aquae*. In the present study after the infection with *A. hydrophila* the liver of infected fish showed deep vacuolisation and granulation in the cytoplasm, necrosis, granulomatous inflammation, and large number of macrophages and fibroblast. But when the fishes were fed with herbal formulated diet the hepatocytes were not degraded and the sinusoids were not dilated. Similar observations were noticed in herbal treated goldfish when the fishes were dipped in aqueous herbal extract. (Harikrishnan *et al.*, 2009) in which the dilation of sinusoids had ceased and formation of normal liver parenchyma occurred. Herbal treatment and histopathological studies were not uncommon. Vinodhini (2009) worked on *C. carpio* exposed to heavy metal induced toxicity, treated the fishes with the extracts of *Nelumbo nucifera* and *Aegles marmelos*. She reported that the treated fishes show decreased edema in the gills and improvement in the recovery of liver damage.

**MUSCLE**

*Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*) were studied by Afifi *et al.* (2000). His studies revealed that corneal spongiosis, ulceration and inflammation of the muscles supporting the sclera were observed. Fatty change, necrosis and pancreatitis occurred in the hepatopancreas. Melano macrophage centre in the spleens were activated (Afifi *et al.*, 2000). Miyazaki *et al.* (2001) reported that the underlying lateral musculature was edematous and possessed markedly atrophied muscle fibers in *C. carpio* fishes infected with *A. hydrophila*. Necrotic changes
of the mucosa together with oedema and presence of bacteria in the muscle layers were observed in turbot *Psetta maxima* infected by *Pseudomonas anguilliseptica* (Magi *et al.*, 2009). In the present investigation also the muscle fibers were atrophied and there was a wider gap between the muscle bundles. Necrosis occurred in control fishes infected with *A. hydrophila*. Carbis *et al.* (1996) detected necrosis or cellular degeneration in the gills, liver and kidney of carp introduced into a tank containing 1.7g/ml of microcystins. Intraperitoneal inoculation caused Tiger Oscar (*Astronotus ocellatus*) infected with motile *Aeromonas septicaemia* contained a large amount of red – acutic fluid accumulated in the abdominal cavity along with haemorrhages in the liver and kidney (Soltani *et al.*, 1998). Histopathological examination of infected *A. salmonicida* fish revealed multifocal hemorrhage and infiltration of lymphocytes and histocytes in sub dermal adipose tissues and muscles. (Thomas Loch and Faisal, 2010). Huizinga *et al.* (1979) reported that focal haemorrhage and dermal lesions accompanied by ulcerative form of the disease were observed in chronic motile *Aeromonas* infection significantly and acute septicaemia was observed in liver and kidneys. In the present investigation also the skin lesions and mild haemorrhage were observed in the fishes exposed to *A. hydrophila*. This shows that the herbs had more influence on the recovery of damaged tissues as the vacuolation and necrotic tissue in the liver and gills are comparatively less in these fishes respond to 1.5% and 2.0% of herbal diets.

**INTESTINE**

*A. salmonicida* translocated through the intestinal epithelium of rainbow trout, suggesting that the intestine is a possible route of infection in salmonids. It is an important step of bacteria invasion. *Aeromonas* produces many virulent factors such as endotoxin, cytotoxin and haemolysins which adhere to erythrocytes and causes
infection (Chopra et al., 2000; Jutfelt et al., 2006). The damaging effects were also described in *Salmo salar* L. infected by *A. salmonicida*, a pathogenic bacteria strain provoked a degeneration to fish intestine (Ringo et al., 2004; Bakken, 2002). Anterior part of intestine in *Ararichas minor* infected by *Vibrio angvillarum* was reported by Ringo et al. (2003). A detachment between muscularis and mucosa layer of intestine was observed in *Mugil cephalus* infected by *A. hydrophila* (Khemiss et al., 2008). Similar observations were also observed in the present study. There was a separation between two layers in the muscle. In aquaculture chemotherapeutic agents such as commercial antibiotics and disinfectants are commonly employed for disease management, although this is not advisable due to cost effectiveness, environmental hazards and the antibiotic resistance developed by many pathogens (Kruse and Soram, 1994). In the present study only moderate lesions were observed in herbal diet fed fishes which were challenged with *A. hydrophila*. The flavonoids present in the plants might have prevented or restricted the entry of bacteria and they antagonize the free radicals and protected the tissues against oxidative stress. As *Withania somnifera* possess good immunomodulatory, anti-inflammatory, antitumor, antioxidant, anticancer properties and many pharmacologically and medicinally important chemicals, such as Withaferins, sitoindosides and various alkaloids, they protect the cells from oxidative damage and diseases (Veena Sharma et al., 2011). In US, the New England Deaconess Hospital, has taken a patent on an Ashwagandha formulation claimed to alleviate symptoms associated with arthritis (Panda and Kar, 1997). In this regard, disease management in fish using phytotherapy may become an attractive alternative, mainly due to the biodegradability of raw materials and lack of side effects (Hobbs, 1994).

Interest on herbal medicine research has been increasing recently. Review of literature also highlights the value of medicinal plants on the fertility improvement with
fewer side effects. *Mucuna pruriens*, recognized as an aphrodisiac in Ayurveda, is known to enhance the functional activities of gonad and secondary sexual organs (Saksena and Dixit, 1987). Further, the *Mucuna pruriens* seed extract brings about a noteworthy increase of sperm population on experimental rats (Amin et al., 1996). The differentiation of germ cells and gonads in the common carp, *Cyprinus carpio* has been investigated by using anticarp sperm monoclonal antibodies (Parmentier and Timmermans, 1985). Fish fed with the control diet and 0.5ml of *Aloe vera* latex/kg diet had exhibited significantly higher and better indices of reproduction traits in Nile tilapia, *Oreochromis niloticus* (Jegede, 2011). The male rats when treated with aqueous extract of *Coriandrum sativum* seeds (250 mg/kg body weight) were active and revealed various stages of spermatogenesis. Fat vacuoles were not apparent in the interstitial tissue, which indicated that these testosterone-secreting cells were active. Similarly, the lumen of epididymis was normal and contained large numbers of mature sperm (Al–Suhaimi, 2008). In Ayurveda, the amazing *Asparagus racemosus* herb is known as the "Queen of Herbs" because it promotes love and devotion. Shatavari is the main Ayurvedic rejuvenative tonic for the female, as is *Withania* for the male. *A.racemosus* enhances folliculogenesis and ovulation (Kalia et al., 2003) and it has estrogenic activity (Tewari et al., 1968). *Asparagus racemosus* (Ar) has been used only clinically and shows oesterogenic effects in adult mammary glands and genital organs of virgin female rats (Pandey and Sahay, 2001). This phytoestrogenic activity is due to the presence of steroidal saponins which exert hormone like actions in the body, and also due to the isoflavones which have mild estrogenic activity that help to balance the estrogen levels. A significant rise in serum follicle stimulating hormones (FSH) is observed in which the female wistar rats were fed with Ar root extract at a concentration of 100 mg/kg (Kalia et al., 2003). In the present study also the
Perinucleolus Follicles, Cortical Alveolus Follicles, Primary Vitellogenic Follicles, Secondary Vitellogenic Follicles and Tertiary Vitellogenic Follicles were observed. A developing oocyte with cytoplasm and a large nucleus, called germinal vesicle were also seen. Prabha et al. (2004) assessed the safety profile of *Asparagus racemosus* by studying acute and chronic toxicity (1g/kg) on pre and post natal development in rats. No changes in liver and renal function test parameters were also reported. The histological studies of liver, kidneys, testis and stomach were also found to be normal. This report suggested that the addition of *Asparagus racemosus* in the diet has no negative effect on the overall development of the animal.

The effects of water extracts of *Cynomorium coccineum* and *Withania somnifera* on ovarian follicular development and serum levels of FSH and LH were studied by Al-Qarawi et al. (2000) in immature 17-day-old and 25-day-old Wistar rats. In 25-day-old rats, extracts of both plants elicited significant changes in gonadotrophin levels coupled with a significant increase in ovarian weight. In the present study several oocyte stages, ranging from primary forms to vitellogenesis, suggested that the maturation was successfully attained and the vitellogenesis show formation of yolk granules in the cytoplasm. These features proved that these three herbs has direct influence on the total development of reproductive system.

This study has shown that these three herbal supplement in fish feeds elicited maximum development in the ovary and the presence of flavonoids and saponins in the plants might have contributed its antioxidant property to fight against *A. hydrophila*. It is therefore beneficial for use in aquaculture to enhance the disease resistant status and reproductive potential of freshwater cultivable fishes.