CHAPTER-III
HISTOPATHOLOGY
INTRODUCTION:

The study of contact, interaction, beneficial or harmful and relationship between the host and the parasite is called as histopathology. Some biologist say that all animals are parasites because they have to rely on other living organisms for their food, be these plant and animals. A parasite is physiologically dependent on its host and cannot survive in its absence. The physiological compatibility is sometimes such that of the host and its parasite may evolve though not necessarily at the same place. Many species of parasite can survive as adult in warm blooded animals and in their larval stages either in cold blooded animals or may be in water and soil.

The host parasite association as it grows older generation after generations, the impact of pathogenicity would theoretically become less and less due to mutual characteristics and biochemical adaptability. The structural and biological characteristics of the parasites, the various modifications in the organs of the attachment. The mechanism of feeding, their life cycle inside and outside the host. The biochemical adaptation of the parasite enabling them to stay alive inside the host. The mechanism of entry and the establishment of a parasite within a particular fishes. The degree of the response by each host to the parasite while making contact is related to the nature of the tissue site of invasion and also to the intimacy of the host or parasite contact. It is also related to the stage of development of the living organisms, whether it is an adult or larval form.

Some parasites are ectoparasite and endoparasite. Ectoparasite living on the surface of the body of their host, such as blood sucking or feeding upon hair, feather skin or secretion of the skin. Endoparasite, living inside the body, occupies digestive tract or other cavities of the body or lives in various organs, blood, tissue, so even within cells. Some parasites pass different phases of their
life cycle in two or more hosts. Parasites have little need for sense organ and seldom have they as highly developed as do related free-living animals.

The metabolic type of the parasite depends on number of factors, including the presence or absence of oxygen in the habitat, on the parasite ability to bind it with its respiratory pigment, on its type of feeding and on its size.

Helminth means ‘worm’. Helminthes includes the animals belonging to the phylum platyhelminthes and nematode of phylum aschelminthes. Many of the parasite form of this group are popularly known as parasite worms. Platyhelminthes is a large and diverse group of organisms, some of which are free-living but most of which are parasite, living on, or in most species of vertebrates and invertebrates. They are flattened dorsoventrally.

Various taxonomical studies revealed that the holdfast organ are ingeniously adopted and this adaptation helps them to attach to the mucosa of the specific host, where as there are other species which are having weakly developed scolex and hence they have a wide range of host specificity.

Cestodes absorb semi digested food material from the intestine and it has long been assumed that cestode lie in a bath of semi digested ‘soup’ from which they can extract nutrients metabolites and invitro. Studies suggest that a complex nutritional relationship occurs between a cestode and its host. The physiological conditions of a specific species depend mainly on the type of sites which available, this may be favourable or unfavourable, whereas the parasite get sufficient nourishment.

Metacestode of tapeworms of the cyclophyllidean family Gryporhynchidae occur frequently in the gut, gall bladder or the abdominal cavity of fish. These cestode stages frequently referred to as ‘cysticerus’ may be more common in host fishes. The histological finding obtained the study show that Neogryporchynthus cheilancristrotus is a truly pathogenic species causing degeneration and inflammation intestinal wall, while the majority of cestode inhabiting the gut of fish live in the lumen of the gut and attached more or less
firmly to the gut epithelium with scolices, *Neogryporchynthus cheilancristrotus* intrudes into deeper layer of the gut wall and breaking through the epithelium is located in the lamina propria of the mucosa layer (Molnar, 2005).

Histopathological investigation such as marked lesions in the mucosa, inflammation and congestion of mucous glands and intestinal hemorrhages lead to severe anemic condition in the fish (Williams, 1967; Roberts, 2001). Besides mechanical injuries, atrophy of tissues and lesions of the alimentary canal, blood vessels or duct etc., the parasite also induce toxic metabolic by product (endotoxic / ectotoxic) eliciting changes in blood, enzymes, vitamins and hormonal activity of the host (Symons and Fairbain, 1963; Poynter, 1966; Mongkol-Primpol, 2000; Ekman & Norrgren, 2003).

The extensive study on the host parasite relationship has been carried out by *Amoebotaenia indiana* by Mitra and Shinde, (1980); *Hymenolepis nana* by Bailey, (1951). Host response to implanted adult *H. nana* was studied by Coleman, DE. Sa (1964) and experimental immunization of dog against *E. granulossus* was first observed by Foresek and Rukavina in (1959). Murlidhar and Shinde, (1987) observed histopathology of *Acanthobothrium uncinathum* from a fish *Rhyncobatus ajeddensis*. Baur *et al.*, (1959); Amlacher, (1961); Hayunga, E.G. (1977) and Mackiewicz, (1972) have studied the histopathology of intestine of fish caused due to cestodes. Gopal Krishnan, (1968); Satpute, A. and Agarwal (1974); Bose and Sinha, (1981), Niyogi, A. and Agarwal, S. M. (1989) have studied the *caryophyllaeidiasis* in fish host.

Smyth, (1969) and Ress, (1967) stated that the response to the adult cestodes only develop of the mucosa of the hosts intestine invaded.

Helminthes parasite poses a serious threat to the fish population. The study of trematode parasite found in fish is a field wide open for investigations always. Although many studies have been made on the trematode in fish, relatively few have been concerned with the histophysiological, histopathological and more particularly the changes in the chemical composition of the host tissues.
(Bose and Sinha, 1979; Barbara, 1980; Muzzal, 1980; Chung Yui-tan, 1981; Christina, 1982; Paperna and Vanus, 1983; Maqbool and Nizami, 1984; Zarina, 1990; and Bhargavi and Krishna, 1993). The parasite may effect host interactions are deleterious to the host (Holmes and Bethel, 1972; Holmes, 1979; Minchella and Scott, 1991). The festation of parasite to the host cause many alterations including the macromolecular compositions of host’s tissues, which is the evidence that was supported by histochemical and biochemical studies.

Mostly digenetic trematodes produce infection in man and animal of the various fluke infections. The minute trematodes attach to the cell in the mucosal crypts, usually at the duodenal and jejunal walls of the small intestine, usually excess secretion of mucus. The damage produced by trematode is mechanical obstructive and toxic. At each site of attachment a mucosal ulcer is produced. In the study of histopathological and histochemical changes that are caused to the intestine of fish *Clarias batrachus* due to infestation of trematode parasite, *Genarchopsis goppo*. Studies have been made to visualize the biochemical changes occurred in fish due to parasitic infestation (Robinson and Williams, 1971 and Gupta and Agarwal, 1984), but relatively a very less information is available on the studies made histochemically, however these studies especially elucidate the changes occurred in the infected tissues of the host due to the parasite.

The pathological consequences of parasite effects on fishes are well documented and parasitic fauna certainly influences the fish health, causes to the mortality and thus reduce fish population. It is well known fact that the helminth parasite bring pathological changes brought by the parasite in the host may be due to the mechanical damage or due to the release of toxins by the parasite.

Gupta and Agarwal, (1984) and Singh *et al.*, (1984) who have also described the pathological condition brought by trematodes in various fishes. *Procamallanus* is a common nematode parasite in the stomach and intestine of fishes inhabiting freshwater, brackish water and marine water
ecosystem of the world (Sinha, 1988; Zaman and Leong, 1988; Chandra, 1994; Mortens and Moens, 1995; Bijukumar, 1996; Gozalez-Solis et al, 2002). Though the intermediate host (vector) of the parasite copepods (Chandra and Modak, 1995; Sinha, 2000), there exist reports that adult as well as larval stages of Procamallanus are pathogenic to fish (De and Maity, 2000; Ruhela et al., 2006).

Histologically larval nematode migration and encapsulation within body tissues and visceral organ often cause the development of lesions described as fibro-granulomatus, although in many instances no host inflammatory reaction is elicited (Fergusen, 1989). Histopathology of infection of Pompophorynchus kashmirensis have been the subject of earlier work at the laboratory (Chishti & Bakshi, 1992; Chishti et al., 2002; Chishti et al., 2003) which provide a convenient fish- helminthes model in which the possible role of acquired immune response was investigated. Voltoneu et al., (1994) described pathological changes due to Raphidasaris acus in liver of roach. Murai et al., (1997) described presence of Paradilepis scolecina larvae in the series membrane of the intestine and gall bladder in beam caught in Lake Balaton. Stephenson et al., (1980) remarked that pathology of the intestinal layer might be due to combination of physical, biochemical and histopathological responses. The pathology of P. laevis infection in the alimentary tract of the salmon (salmo salar), the club (Leuciscus cephalus), the rainbow trout (Salmo gairderi) and the stone loach (Nemacheileus barbatulus) was demonstrated in earlier studies by Dezfuli B.S., 1991; Hine, P.M. and Kennedy, C.R. 1974; Pipy, J.H.C. 1969; Wanstali S.T. et al, 1986).

The present study deals with the investigation on the Senga rupchandensis n. sp., Circumoncobothrium jadhavae n.sp., Genarchopsis paithanensis n.sp., Allocreadium khami n sp., Orientocreadium striatusae n. sp. collected from freshwater fishes namely Channa striatus (Bloch, 1793) and Mastacembelus armatus (Lecepede, 1800).
MATERIAL AND METHODS:

For the histopathological study, the freshwater fishes, namely *Mastacembelus armatus* (Lecepede, 1800) and *Channa striatus* (Bloch, 1793) were collected from the different region of Marathwada (M.S.), India, and brought to the laboratory and killed by pithing brain they were examination externally (i.e. scale, gills, fins) and later cut opened the fish and observed internally taken out the intestine, liver, heart, spleen in the normal saline water in petridish and cut opened; examination carefully for parasites. The cestode were collected from the intestine, liver and trematode worms were collected from intestine, stomach, liver, gill cavity etc. identified worm were kept separately and wash in saline water solution, flattened by using coverglass and slide then preserved in 4% formalin for taxonomical studies. The slides were prepared by Harri’s Haematoxylene stain, dehydrated in alcoholic grades (30%, 50%, 70%, 90%, and 100%), cleared in Xylene and mounted in DPX. Drawing were made with the aid of Camera Lucida, all measurement are taken in millimeter, identification was carries out by the using Systema Helminthum cestode Vol-I (1956), and trematode Vol- I and II (1971) by Yamaguti.

The infected intestine, liver attached with the cestode parasite and infected liver, stomach, intestine, gills with attached trematode parasite, were kept intact and small pieces of such intestines, stomach, liver, gills were fixed in Bouins fluid for histopathological studied. The fixed tissues were washed in distilled water, dehydrated in alcoholic grades, cleared in xylene, embedded in paraffin wax with melting point (58-60 °C).

Block were cut at 8mµ and slides were stained with Haematoxylene counter stained with eosin stain. Best slides were selected, observed under the microscope and photographs are taken.
RESULT AND DISCUSSION:

The histopathological study is carried out with microtome technique, where as the section were cut 8µ on a rotary microtome and stained with Harri’s Haematoxyylene counter stained with Eosin. The best select slides of healthy and infected intestine, gills and liver was observed in the microscope and photograph of the best slides were taken and are considered for the discussion.

*Senga rupchandensis* n.sp.

The taxonomical observations reveals that, the present worm belongs to the genus *Senga* as new species i.e. *Senga rupchandensis* n.sp. The worm along with intestine is utilized for the histopathological study.

On closer observation of the transverse section of healthy intestine of *Channa striatus* (Bloch, 1793), the serosa, muscularis, sub-mucus and mucosa layers of intestines were clearly observed which are intact and healthy (normal) (Plate-20, Fig. 1) whereas in the infected intestine with the cestode *Senga rupchandensis* n. sp. it is observed that the worm has high penetrative type of scolex and they cause heavy mechanical tissue damage to their host. The colour changes of the infected intestine from whitish to yellowish.

Histopathology caused by the parasite pierced to the intestinal tissue makes the hole or vacuolization with the help of scolex.

In the transverse section of infected intestine with the *Senga rupchandensis* n. sp. was clearly observed that, the worm penetrates through the intestinal villi and ruptured the mucosa and sub-mucosal layers. The parasite penetrates deeply through the intestinal tissue layer and causes heavy mechanical injury by the penetrative type of scolex (Fig. 2). The worm tried to obtain all the nourishment from the host. The effect of parasites on the get serious of change which ultimately reduces the absorption rate and interrupts the other metabolic processes. The biochemical studies show the changes that occur in the infected
tissues including the change of carbohydrates, lipid, protein nucleic acid and enzyme levels.

The present study showing that, the worm *Senga rupchandensis* n. sp. is having penetrative type of scolex and is highly pathogenic parasite to the host, *Channa striatus* (Bloch, 1793). This pathological result is resemblance and discussed with Satpute and Agarwal, (1974a) also noticed inflammatory response in submucosa and serosa of *Clarias batrachus* infected with *Lytocestus indicus* and *Diphyllobothrium penetra*.

Karanis and Taraschewski, (1993), described the histopathological changes in cyprinids infected by *Caryophyllaeus laticeps*, the scolex of *Caryophyllaeus laticeps* cause only local compression of the hosts gut epithelium and at the site of attachment of these cestodes vacuolation of epithelial cells and ruptures the brush border.

Hiware, C.J. (2002) has studied the pathological changes of intestine of *Clarias batrachus* infected by the *Lytocestus maldurgensis*, the scolex deep penetration through the intestinal villi, totally breaks or damage the sub-mucosal, mucosal, muscularis. The scolex parasite comes to lie near the serosa layer.

Molnar, K. (2003) has studied the histopathological changes in common carp, *Cyprinus carpio* (L.) infected with *Atractolytocestus huronensis* and shown that the cephalic part and neck of the *Atractolytocestus huronensis* is found in the deeper layer of the intestinal mucosa, the scolex was surrounded only by the basement membrane which separated it from the lamina propria of the intestinal mucosa, worm penetrating into the deep layer of the mucosa containing intestinal crypts and damage of the epithelium layer.

Molnar, K. (2005) shown that the cestode parasite, *Neogryporhynchus cheilancristrotus* infected to *Gibel* carp fish. Cestode parasite is a truly pathogenic species causing degeneration and inflammation in the intestinal wall, their scolices of *Neogryporhynchus cheilancristrotus* intrudes into deeper layer
of the gut and breaking through the epithelium is located in the lamina propria of the mucosa layer.

**Circumoncobothrium jadhavae n.sp.**

On closer observation of the transverse section of normal (healthy) liver of the *Mastacembelus armatus* (Lecepede, 1800) was clearly observed in the microscope (Plate-21, Fig.1) whereas in the infected liver with cestode *Circumoncobotrium jadhavae* n.sp. cysts damage the liver tissue. In the infected liver change the color from reddish to yellowish.

Histological examination of the infected liver with *Circumoncobothrium jadhavae* n.sp. cysts is attached into the middle portion of the host liver. This cysts is double layered with coiled the larvae in the cysts. The macrophages gathered around the cyst wall, the necrosis of parenchyma cells immediately around the cysts is evident and also large number of inflammatory cells around the cyst, blockage of bile passages, enlargement of hepatocytes and liver vacuolation from the cyst, the sinusoid were ruptured and filled with blood (Fig.2).

The changes that are caused in the host livers are due to the mechanical damage that may be due to the release of toxins by the parasites. The parasite not only change the morphology of the host, but also interfere with the nutrition and metabolism and disturb the movements and secretory functions of the alimentary canal and its associated glands, the influence of the parasite on the host is adverse.

The present study showing that, the histopathology of infected liver with *Circumoncobothrium jadhavae* n.sp. cyst. This results are matching in accordance with the study carried out by Raymond C. Bowen, (1969) have studied the histopathology of plerocercoid of *protocephalus sp.* infected to the liver of *Lepomis macrochirus*. Each larval tapeworm was surrounded by a cellular capsule containing spindle shaped cells with elongated nuclei. Liver cells
adjacent to the capsule appeared more vacuolated than of the parenchyma. Paul C. Stromberg and John L. Crites, (1974) has studied the histopathological changes in liver of the fish *Marone chrysops* infected by the larval *Trianenophorus nodulosus*. A distinct cellular response in the liver, destruction of the proximal liver parenchyma, compression pancreatic tissue, destruction of the squamous, metaplasia and fibrosis. S. Radhakrishnan *et al.*, (1983) has studied the histopathology of marine teleost fish, *Saurida tumbil* (Bloch) liver infected with cestode *Penetrocephalus ganapati* cyst. Scolex was embedded and encysted in the liver and neck connecting the scolex with strobila its major part lies in the viscera, the ensheathed neck separate and take independent paths so that the scolices are encysted seperately inside the liver.

Ewa Dzika *et al.*, (2005) have studied the histopathology of the liver of *Ratilus ratilus* L. infected with the *Paradilepis scolecina*, resulted changes in the liver, vacuolar degeneration of liver cells, dispersed foci in liver parenchyma, large melano-macrophage centre and necrosis was accompanied by focal infiltration of lymphoid cells.

**Genarchopsis paithanensis** n.sp.

On closer observation of the transverse section of healthy intestine of host, *Mastacembelus armatus* (Lecepede, 1800) all layers are clearly observed (Plate-22, **Fig.1**), whereas in the infected intestine with trematode parasite, *Genarchopsis paithanensis* n. sp. causing damage to the epithelial layer.

*Genarchopsis paithanensis* n. sp. trematode is found in the anterior part of the intestine. Longitudinal section of the trematode parasite infected with the intestine of *Mastacembelus armatus*, the anterior end of the trematode parasite *Genarchopsis paithanensis* n.sp. was approaching the intestinal villi (**Fig.2**) and damage the epithelial layer, embedded in the fibroblast, lymphocytes, plasma cells and attached to the intestinal villi, therefore, causing inflammation, vacuolation and damage the intestinal villi (**Fig.3**).
The worm is not only successful to enter into the intestine forming the ulceration in the intestinal wall causing damage to the host tissue but the parasite may affect host physiology in many ways that induce stress in the host. The parasitic infection to the disturb the circulation of sugar levels which in turns effects other metabolic pathways.

The present study showing that, the *Genarchopsis paithanensis* n.sp. damage the epithelial layer, this result are matching in accordance with the studies carried out by Barbara, (1980) who has studied the pathological changes brought by *Bucephalus polymorphus* and *Rhipidocotyle illensis* in cyprinid fry. The damage caused to a white sucker fish by the trematode parasite *Triganodistomum attenuation* was reported by Muzzal, (1980). In contrast, Banergee G. *et al.*, (2006), has studied the histopathology of the intestine of freshwater fish, *Clarias batrachus* infected by the trematode parasite, *Genarchopsis goppo* caused extensive damage to various layer of the intestine right from mucous membrane to muscularis layer, extensive damaged caused to the villi.

*Allocreadium khami* n.sp.

On closer observation of the transverse section of normal (healthy) liver of *Mastacembelus armatus* (Lecepede, 1800), the liver cell, hepatocytes are clearly observed (*Plate-23, Fig.1*), whereas in the infected liver with the trematode parasite *Allocreadium aurangabadensis* n.sp. cysts damage the liver tissue, vacuolization and change the shape and size of liver.

Histopathological examination of infected liver with *Allocreadium khami* n.sp. trematode shows that cysts were attached to the serosal coat of the liver and damage the liver tissue. The cyst is large with parasite and filled with eggs. The macrophages scattered around the cysts, distinct shape of hepatocytes, vacuolization and damage the liver cell or necrosis is seen with (*Fig.2*), sinusoid were ruptured. The infection in the liver by a parasite causes disturbances in the
vital functions of the glands. These disturbances may directly affect the chemical nature of the infected tissue by lowering or increasing the important molecules which plays important role in metabolism.

Liver is the one of the important digestive glands. It is the chief organ of the process of detoxification. It plays a role in the metabolism of carbohydrate, protein and lipid, storage of glycogen, desaturation of fatty acid, amino acid carried out by the liver (B.Lakshman Reddy, 2006).

The result of the present study are similar in accordance with the studies carried out A.J. Mitchell, (1982) who studied the histopathology of the liver of Fathed minnow (Pinephales promeleas) fish infected with Posthodiplostomum m. minimum trematode encysted in liver tissue causing little damage to hepatocytes, melanin-macrophage centres were diffusely scattered throughout the fibrinous, fibroblast produce containing collagenous connective tissues. Reddy B. Lakshma et al., (2006) have studied the histopathological in liver of freshwater fish, Channa punctatus infected with the Euclinstomum heterostomum includes enlargement of hepatocytes, vacuolation of cytoplasm, disarray of hepatochond, hypertrophy of hepatocytes and liver vacuolation. The present study is in contrast from result of J.E. Revenga et al., (2006) have studies and observation of the histopathology of the liver of Galaxias maculates fish infected parasites found in the hepatic parenchyma of “puyenes”, causing hepatic parenchyma was unaltered a distance from the foci of the lesions and weak inflammatory reactions.

**Orientocreadium striatusae n.sp.**

On closer observation of the transverse section of healthy buccopharyngeal tissue of the host, Channa striatus (Bloch, 1793) it located posterior region of the head are clearly seen (Plate-24, Fig.1), whereas buccopharyngeal area infected with trematode Orientocreadium striatusae n.sp. cysts embedded between the pharynx and gills causing inflammation of
buccopharyngeal wall (Fig.2). Externally observed, changes the colour of infected gills from reddish to whitish. Gills are the very important part for the respiration in fishes.

In the transverse section of the infected buccopharyngeal area with Orientocreadium striatusae n. sp. the trematode cysts is large and surrounded by dense connective tissue, inflammation of the buccopharyngeal area. Parasite causes breaks and destroys the fat tissue of the host. Blockage the gill capillaries, lamellae and prevent the blood circulation as well as respiration of the host (Fig. 2, 3).

The present study showing that, the Orientocreadium striatusae n.sp. damages the buccopharyngeal area of the host, Channa striatus (Bloch, 1793). This result discussed with Wallace A. Evans, (1974) have seen the histopathology of gill arches of Cutthroat trout (fingerlings) infected with blood flukes, Sanguinicola klamathensis. These parasite eggs occurred more frequently in the capillaries of gills, changes in gills, hyperplasia, and chronic inflammation of the gill epithelium and fusion of lamellae. Baur O.N. et al., (1959) indicated that the blocking of gill capillaries by Sanguinicola sp. eggs causes necrosis of the gills. Schaperclaus, W. (1954) indicates that the eggs of Sanguinicola intermis infected to the fish causing they clog of gill capillaries bring about thrombosis and death the fish. Robert E. Olson et al., (1997) have studied the histopathology of gills of Steelhead trout (Oncorhynchus mykiss) infected with the trematode metacercaria caused the hyperplasia of filament epithelium, in some areas did lead to fusion of gills lamellae and loss of respiratory surface. Jorge da Costa Eiras et al., (1999) have studied the histopathology of gill arches infected with the Clinostomum marginatum cyst. In the gill arches the dense connective tissue capsule surrounding the parasite causing the fat tissue destroyed, its all completely broken, the typical architecture of the tissue was changed into an irregular mass containing a great amount of cell remnant and debris, tissue destruction from the cyst.
Jirawat Tudkaew et al., (2008) have studied the histopathology of gill of the Orange spotted grouper (Epinephelus coioides Hamilton, 1822) it is marine fish infected with Ganapodamium epinephili trematode cyst showed the parasites were tightly packed underneath the mucosal layer of the primary gill lamellae, secondary lamellae have disappeared, the cyst wall was composed of epithelial cells of primary gill lamellae, parasitic cysts expanded and destroyed the secondary lamellae and small suckers embedded underneath of the gill epithelium.

CONCLUSION:

It is concluded that, the parasites are very dangerous for human being as well as other animals. Fishes gives content of protein, provides vitamin A, vitamin D and as a commercial economic point of view and useful for preparation of soup, liver oil. The study of the helminth parasites are necessary because the parasite affects on productivity of the fish population which may cause deterioration in their food value, by decreasing growth rate, reducing the quality of flesh, loss of protein, behavioral changes as well as reduces the absorption and metabolic process result may in heavy mortality. Besides, infected fishes act as a very potent source of helminth infections of man and they transmitted (to man) only through eating of fish.