CHAPTER NO. 4

HISTOPATHOLOGICAL STUDIES

INTRODUCTION

All scientific investigations are essentially a search for truth based on meticulous experimentation keen and careful observation of results of the experiments and widely acceptable conclusion.Experimentation is a vital factor in scientific investigation.For all scientific investigations certain techniques are necessary.Practically in all fields of biological studies and research, the preparation of material from living tissues for study under microscope is very essential. Therefore, for many biological studies and researches, for pure and applied histochemical studies and for purely clinical and pathological investigation, a technique which provides a disciplined laboratory process for the preparation of the material (slide) from the tissues , for study under microscope is necessary. This process of preparation is called microtechnique. The science of microtechnique may be called a social art. In medical research which is the life saving adventure, the study of cells and tissues has always been important. The person who prepares the microtechnique material for such study is responsble for the success and effectiveness of the research. Thus the role of the microtechnicians in biological and pathological laboratories is basically vital.Microtechnique enables us to study the tissues in their original condition with the least amount of postmortem changes involved. This technique provides a continuous series of sections for studying not only a part of the tissue but the entire tissue microscopically for various purposes. It provides us a way to study the individual cells and also many different cells (tissues) simultaneously. The study of sections gives us a picture of interrelationship of structures amongst the tissues therein. Through this study we can completely reconstruct any strucuure under observation. Microtechnique provides us a ground to correlate the tissue structure with the function. It provides an opportunity to interpret the physiological role of the structural elements of the tissues.For different

studies the tissue of organ under medical examination is removed from the body this removal is called biopsy. Microtechnique is the basic way to study and diagnose the diseased conditions in cells (cellular pathology), diseased conditions in tissues (histopathology). Histopathology is a well known technique to understand relationship between the concentration of toxicant in the water and its effect on the target organs of the fish. It also gives us an idea about the safe concentrations of chemicals in the environment. It can be used to determine the effects of environmental contaminants on fish and other aquatic organisms. Histopathology can be used to study complicated problems in the aquatic toxicology; identification of toxic mechanisms and prediction of safe concentrations.With the help of histopathology we are able to trace the mode of action of a toxicant on the fish by observing the change in the structure of target organ e.g. cells, tissue, organs and function of the organ.

Histopathology has been accepted as an important tool in medicalscience for many years.“Cellular Pathology”,“Histopathology”,“Structural Pathology” of Vichow has become a corner stone in the field of biomedical Pahtology (Rather 1971). In addition to this important role in biomedical pathology, histopathology is also used extensively in bio-medical toxicology. It is accepted as one of the most important determinants for establishing acceptable concentrations of environments, drugs, food additives consmetic additives and other chemicals. In contrast the value of histopathology in aquatic toxicology has yet to be fully established. In the past 10-15 years a number of reports have been published on histopathology to determine the effects of environmental contaminants on fish and other aquatic animals.

Histopathology techniques can be used in contaminant studies to solve two interrelated problems in aquatic toxicology. Identification of toxic mechanisms and prediction of safe contaminant concentrations. The primary objective of the studies designed to identify mechanisms of toxicity is to describe the mode of action of a toxicant on an organism by identifying the structure (e.g.target organs, cells or organelles) and the functions (e.g.respiration, reproduction, ion transport etc) that the toxicant affects directly. The primary objective of the studies is designed to predict "Safe" contaminant concentrations in laboratory, the concentrations of toxicants that will not adversely affect populations or communities in the environment.

MATERIALS AND METHODS

Procurement and maintenance:

The fish B.carnaticus were collected from Girna dam which is constructed on the Girna River near Nandgaon in Nasik district. The fishes were brought to laboratory and were released in glass aquaria. The fishes were fed on insect larvae and allowed to acclimatize to laboratory conditions for one week. Water was aerated twice a day to prevent hypoxic conditions.

To conduct the histopathological studies, fish of uniform size, approximately 100 gm were exposed to sub lethal concentrations of Atrazine for 96hrs. The lethal concentrations of Atrazine, Contaf and Fenvalerate was 6.521ppm, 3.9711ppm, 3.6271ppm for 96 hours respectively. The fishes were divided in experimental and control groups. 10 fishes were exposed to sublethal dose of Atrazine, Contaf and Fenvalerate respectively. Then dead fishes were immediately removed. 10 fishes were used as a control and kept in water without any treatment. After completion of exposure in sub lethal concentration and control group, fishes wereweighed&then dissected; liver, gonads and kidney were removed. Liver and gonads were weighed and all the tissues were further processed for histology.

Fixation: After dissection the organs like liver,gonads and kidney were fixed in Bouin's fixative for twenty four hours to immobilize the cell structures while maintaining their morphological identity. Fixatives prevent the autollysis, renders proteins of the tissue insoluble and converts living or fixed natural jelly like condition of the cell into fine granular spongy mass. The change that occurs during the fixation is denaturation of proteins,it changes the texture and reactivity of the tissue. The aqueous Bouins fluid is used for the fixation of tissues and it consists

of saturated solution of Picric acid 75 ml, Formaldehyde 20 ml, Acetic acid 05 ml. After fixation, the gonads, liver and kidney were washed under running tap water at room temperature to remove traces of the picric acid as it can hinder the staining process.

DEHYDRATION -After the period of fixation of tissue is over, the fixed tissues were dehydrated to remove water from the tissues. During the dehydration gradual removal and replacement of water from the tissues is doneand for that purpose progressive higher grades of alcohol 30%, 50%, 70%, 90% and 100% that is absolute alcohol is used and the dehydration of tissues by using the graded alcohol is very significant because it prevents the putrefaction of the tissues. It disallows the cellular deformities and cell membranes of the cells are protected from damage. Tissues were placed in absolute alcohol for ten minutes, with one change of fresh absolute alcohol aftertwo minutes. Dehydration was also done at room temperature.

CLEARING OF TISSUES – The process of dehydration leads to the saturation of tissues with alcohol. After dehydration the tissues were impregnated with paraffin wax to make it firm for the purpose of section cutting. This means the paraffin has to remove the alcohol from the tissue to take its place. However the diffusion of paraffin into the tissue to replace the alcohol is not possible as the paraffin is immiscible with alcohol. Therefore , after dehydration the tissue has to pass through an intermediate step in which it is placed in a fluid, which being miscible with alcohol and paraffin ,makes the paraffin to infiltrate into the tissue. This intermediate step is called clearing. The clearing of the tissue is done by clearing agent xylene, which is commonly used as a clearing agent. It brings about the infiltration of paraffin into the tissues but also make them transparent by removing their opacity. Tissues were kept in xylene

for ten minutes.Two changes of xylene were given at an ten minutes interval. After this they were kept for cold infiltration in a mixture of xylene and wax (at room temperature) for two hours.Xylene bring about the infiltration of paraffin into the tissue and also make tissue transparent.

EMBEDDING AND BLOCK MAKING – The embedding is essential to standardise the tissue for microscopic examination by sectioning with microtome. For embedding paraffin wax is used because of its certain unique properties. It is a mixture of aliphatic hydrocarbons. During the hot infiltration the tissues were soaked in molten wax at a standard temperature coinciding with the melting point of the embedding medium used.Hot infiltration in hot wax for ten minutes with two changes of molten paraffin wax of 52^{o}c -54^{o}c. All tissues given three to four wax changes for better impregnation. After the tissue embedded with wax they were cast into a block of paraffin. All type of care is taken for proper reinforcement of wax on all the sides of tissues during block making.Blocks were prepared, trimmed and kept overnight. Sections were cut at 7 microns on the rotary microtome.

STAINING

HAEMATOXYLIN AND EOSIN (HE):

1) Sections were deparaffinezed in xylene and brought down to water via alcohol grades.
2) They were stained in Delafield's haematoxylin.
3) Then they were washed under running tap water for fifteen minutes.
4) They were then dehydrated via graded alcohol to 90%.
5) They were counterstained in 0.49% Eosin (Gurr) in 90% alcohol.
6) Slides were rinsed in two changes of 90% alcohol.
7) Dehydrated in two changes of absolute alcohol.
8) They were then cleared in two changes of xylene of half an hour duration each and mounted in DPX.

RESULTS AND OBSERVATIONS

Atrazine, Contaf and Fenvalerate induced histopathological changes in liver, kidney, testis and ovary of B.carnaticus were observed as follows : -

Liver: Control (Plate -1, Fig.1)

The histology of the liver of the control fish,B.carnaticus showed the presence of hepatic cells (hepatocytes)which were polygonal in shape, each with a centrally placed rounded nucleus with distinct nucleolus and filled with fine and clear cytoplasm.The liver is however not divided into hepatic tubules. The hepatic cells are arranged in cords.In many places sinusoids were seen, in which the blood cells observed distinctly, because liver is haemapoetic organ. A few bile ducts were also seen and they are lined by cuboidal cells on the inner side, surrounded by a layer of fibrous connective tissue of cell types, e.g. kufper cells, fat storing cells, macrophages and pericytes. The section also reveals the presence of pancreatic tissue embedded in the liver (Vasait,2002).

Experimental liver

When the liver is treated with sub lethal dose of Fenvalerate for 96 hours it showed appearance of small vacuoles, degeneration of hepatocytes and proliferations of ducted cells, necrosis is observed in liver (Plate-2,Fig.2). These results are in agreement with these of Saxena et al. (1989). They reported that Malathion is more toxic than carboryl and the denova synthesis of lipid in the liver of Channa punctatus.

After treatment with the sub lethal dose of Atrazine for 96 hours the liver shows appearances of small vacuoles , proliferations of ducted cells, hepatic cells are scattered and showed large vacuoles, in many places and necrosis.(Plate-9, Fig.1) It also shows dgeneration of hepatic cells caused by displacement of nuclei. Disarray of hepatic cords is seen.

Similarly the liver after treatment with sub lethal dose of Contaf for 96 hours showed appearance of small vacuoles, degeneration of hepatocytes and necrosis of cells, proliferations of ducted cells, necrosis. (Plate-15, Fig.3).

Control Kidney- (plate-2, Fig3)

General histology: - Teleostean kidney consists of head and body. Head kidney is the anterior portion of the kidney and consists of lymphoid tissue. The interstitial tissue is the major hematopoietic tissue in the body. Each nephron consists of two parts, the glomerulus and the urinary tubule. The glomerulas capsule consists of an inner layer of single flattened epithelia. Renal tubules consist of single layer of epithelial cells. Mesangium fills the space between the loops of glomerular capillaries. Renal tubules are thin and short in the neck segment. The proximal convoluted segment is divided into two parts i.e. segment I and II. The renal tubules are composed of cuboidal epithelial cells with densely arranged microvilli in the tubular lumen. In segment II, renal tubules are composed of cuboidal epithelial cells. Cilia and microvilli are found in the tubular lumen. In the distal convoluted segment, epithelial cells have no microvilli. The kidney of control fish shows the number of coiled uriniferous tubules. Columnar epithelial cells with nucleus characterize the proximal segment. The nuclei are rounded and prominent. The distal tubules are surrounded by an alkalinement membrane. Interspaced

between the tubules are haemopoietic tissues. The glomeruli also present in the transverse sections, and it showed many nephrons and each nephron consists of two parts the glomerulus and urinary tubule, normal distinct glomerulus with proximal tubule, conducting tubule with connective tissue.

Experimental Kidney :

When it is exposed to sub lethal concentrations of Fenvalerate, it shows degenerative changes in epithelial cells of proximal tubules and haemopoetic tissues, severe necrosis in the proximal tubules leading to the formation of vacuoles, degenerative changes in epithelial cells of collecting tubules (Plate-4,Fig.1).When it is exposed to the sublethal dose of Atrazine it shows disorganized Proximal and distal tubule and sinus appeared in connective tissues (Plate-10, Fig.3). When it is exposed to sublethal dose of Contaf it showed damaged proximal and distal tubule and sinus appeared in connective tissue, necrosis, swelling in renal tubules etc.(Plate-17,Fig. 2)

When kidney is exposed to sub lethal doses of Atrazine, Contaf and Fenvalerate for 24, 48, 72 and 96 hours stage then histology of experimental kidney showed damaged structure and it is dependent on concentration and duration of exposure.

Control ovary: (Plate-4, Fig.2)

Ovary: Ovaries are pair of compact bodies which remain in their original position in the abdominal cavity attached to the dorsal body wall by a fold of peritoneum. Thick ovarian wall with increased vascular supply and conspicuous blood capillaries. The connective tissue in the stromal was evident in good volume. A large number of oocytes stage I,

few oocytes stage II and it shows progressive ovarian development with thining of ovarian wall. The germ cells become associated with small epithelial cells more into cortex. The associated epithelial cells multiply and surround the germ cells which is now called oocyte developing into the stage I, stage II, stage III etc. and they will develop into the mature ovum which is nourished by the surrounding follicular cells.

Experimental ovary:

When the B.carnaticus exposed to the sublethal dose of Fenvalerate for 24, 48, 72 and 96 hours respectively it showed maximum loss of architecture at 96 hrs. That is disrupted follicular cells. Nucleolus showed condensation of crescent shaped dark granules at one side. Degeneration of epithelial cells causes vacuolation, breakdown of germinal vesical, many disrupted oogonia appeared(Plate -5,Fig.3).

Similarly when the B.carnaticusexposed to the sublethal dose of Atrazine for 24,48,72 and 96 hours respectively, then the ovary showedsignificant changes in architecture at 96 hours exposure. Ovary affected by sublethal dose of Atrazine showed destroyed and disrupted follicular epithelial cells. Nucleolus showed condensation of dark granules at one side, vacuolation at periphery of oocyte. (Plate-12,Fig.2). Effect of pesticide on the ovary is concentration and duration dependent process. Similarly when ovary is exposed to the sublethal dose of Contaf for 24, 48, 72 and 96 hours respectively.Maximum loss of architecture is seen at 96 hours exposure.(Plate-19, Fig.1).It showed degeneration of oocytes and atresia. It also show destruction of maturing yokly oocytes because of which ovary reduced in structure. Inhibition of growth of oocytes, especially the atretic follicles was observed.

Control testis (Plate -6, Fig.1)

Histology of normal testis shows the presence of healthy seminiferous tubules, which is internally lined by tubular epithelium which gives rise to spermatocytes. Testis of control fish were composed of lobules showing active spermatogenesis. Sperm nests were found in majority of lobules. Spermatids were the pre dominant followed by dividing spermatocytes and secondary spermatogonia. Majority of the spermatogonic nests were in active state of division. The peripheral part of lobules had a normal healthy structure. Sertoli cells were normal with granular cytoplasm containing a large nuclei with a peripheral nucleolus. In a number of places the inter lobular septa were marked by the presence of small and large aggregations of polygonal leydig cells. They have large nuclei with granualar chromatin and two or more nucleoli.

Experimental testis

As compared to control testis of fresh water teleost fish B. carnaticus treated with sublethal dose of Fenvalerate for 24, 48, 72 and 96 hours.Mximum damage is produced at 96 hours that is disrupted seminiferous tubules and immature spermatogonia and general inflammatory response is observed(Plate -7,Fig.2). Similarly when it is exposed to sublethal dose of Atrazine for 24, 48, 72 and 96 hours it showed significant changes in the number and condensation of spermatogonic cells as well as inflammation of cells, contratctionand vacuolation of tubules.(Plate -14, Fig.1). When it is exposed to sublethal dose of Contaf for 24,48,72 and 96 hours. It showed disorganization of spermatogonia and degeneration of spermatids. The interstitial component contain small cells, less cytoplasm.(Plate-20, Fig.3).g

ABBREVIATIONS

PLATE NO. 1

Fig. No. 1

HC : Hepatocyte, S : Sinus, N : Nucleus

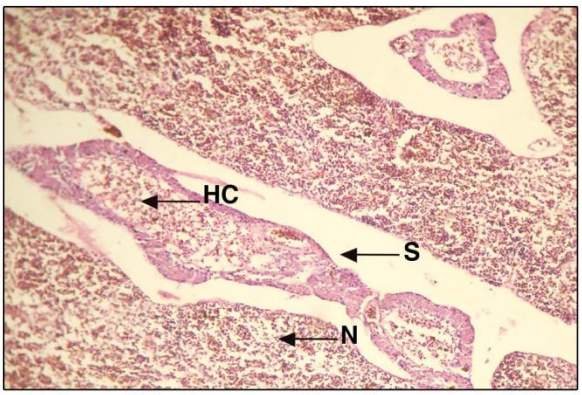
Fig. No. 2

HC : Hepatocyte, S : Sinus, N : Nucleus, HE : Haemorhage

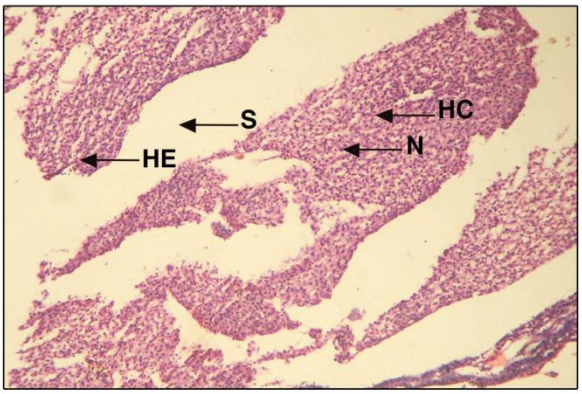
Fig. No. 3

HC : Hepatocyte, S : Sinus, N : Nucleus, HE : Haemorhage

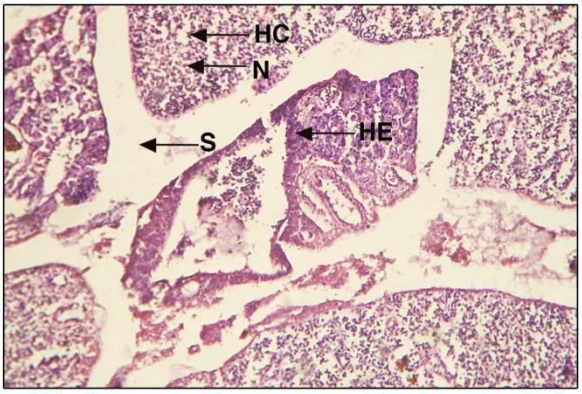
PLATE - 1



T.S. of Liver of *Barbus carnaticus* from control fish 200x



T.S. of Liver of *Barbus carnaticus* exposed to Fenvalerate for 24 hrs. 40x



T.S. of Liver of *Barbus carnaticus* exposed to Fenvalerate for 48 hrs. 200x

ABBREVIATIONS

PLATE NO. 2

Fig. No. 1

HC : Hepatocyte, S : Sinus, N : Nucleus,HE:Haemorhage,NC:Nucleous,BC:Bile cells.

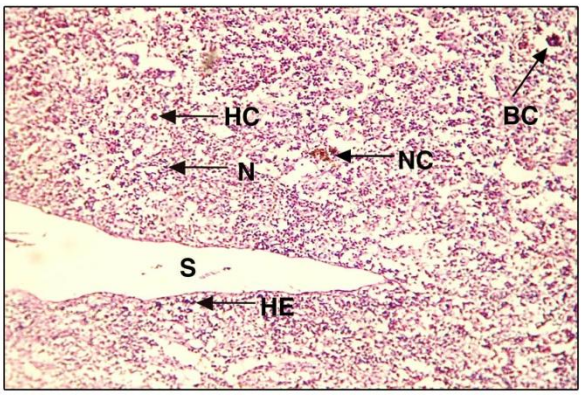
Fig. No. 2

HC : Hepatocyte, S : Sinus, N : Nucleus, HE : Haemorhage,NC:Nucleous, VC:Vacuolization,k:

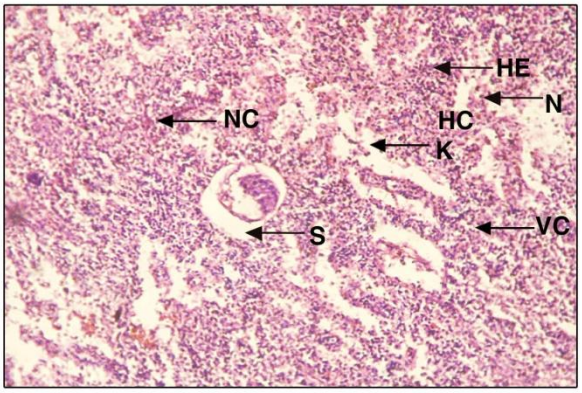
Fig. No. 3

GL:Glomerulus, UT:Uriniferous tubule, BC:Blood capillary.

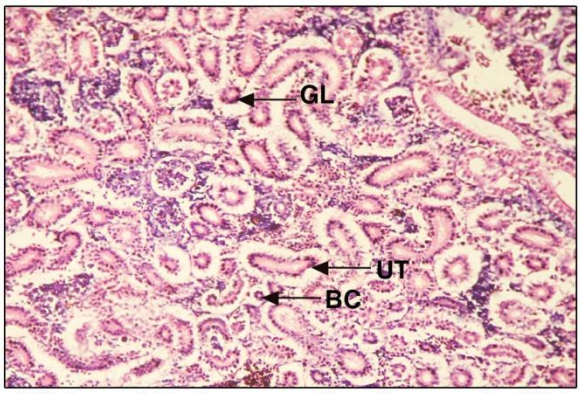
PLATE - 2



T.S. of Liver of *Barbus carnaticus* exposed to Fenvalerate for 72 hrs. 200x



T.S. of Liver of *Barbus carnaticus* exposed to Fenvalerate for 96 hrs. 200x



T.S. of Kidney of *Barbus carnaticus* from control fish 200x

ABBREVIATIONS

PLATE NO. 3

Fig. No. 1

GL:Glomerulus, UT: Uriniferous tubule, CT: Connective tissue.

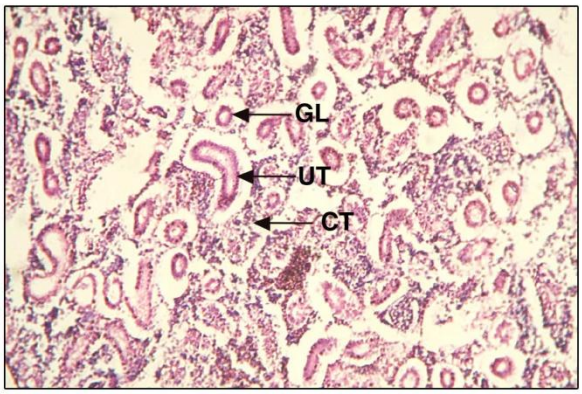
Fig. No. 2

GL:Glomerulus, UT: Uriniferous tubule, CT: Connective tissue.

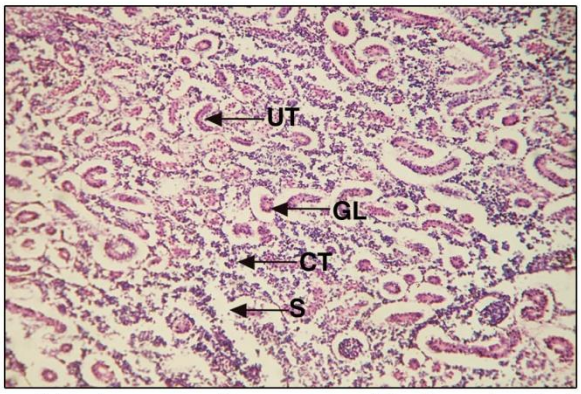
Fig. No. 3

GL:Glomerulus , UT: Uriniferous tubule, CT: Connective tissue.

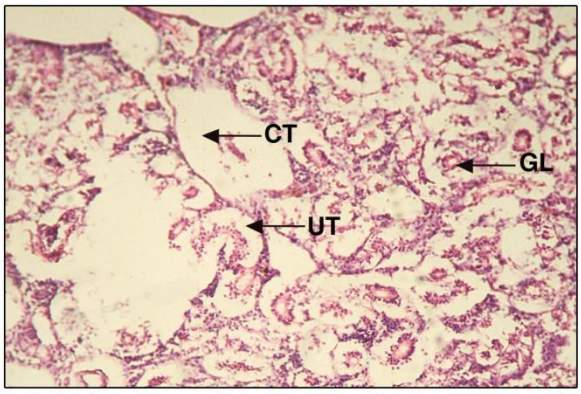
PLATE - 3



T.S. of Kidney of *Barbus carnaticus* exposed to Fenvalerate for 24 hrs. 200x



T.S. of Kidney of *Barbus carnaticus* exposed to Fenvalerate for 48 hrs. 200x



T.S. of kidney of *Barbus carnaticus* exposed to Fenvalerate for 72 hrs. 200x

ABBREVIATIONS

PLATE NO. 4

Fig. No. 1

GL:Glomerulus,UT:Uriniferous tubule, CT: Connective tissue.

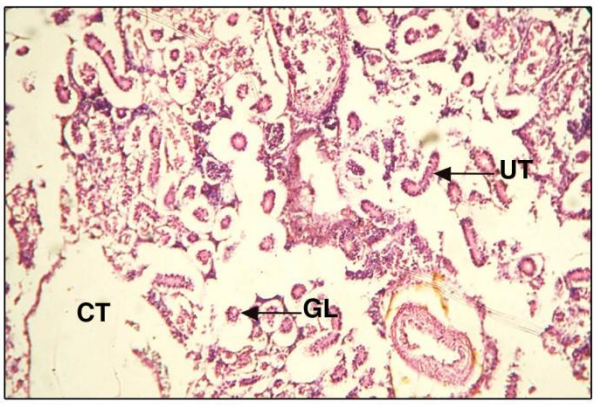
Fig. No. 2

OC : Oocyte, OW:Ovarian wall, FE:Follicular epithelium,N:Nucleus,NU:Nucleous.

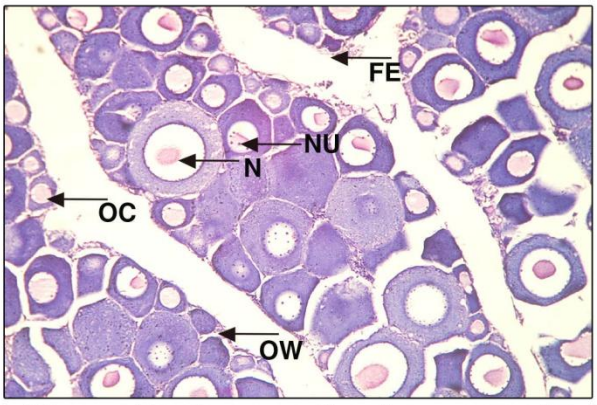
Fig. No. 3

OC:Oocyte,OW:Ovarian wall,FE:Follicular epithelium, N:Nucleus, NU:Nucleolus,CT:Connective tissue.

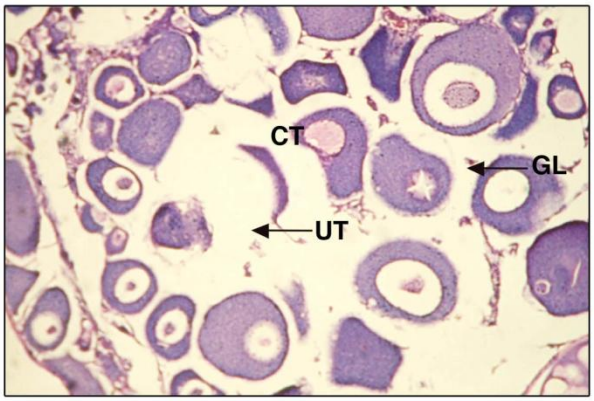
PLATE - 4



T.S. of Kidney of *Barbus carnaticus* exposed to Fenvalerate for 96 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* from control fish 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Fenvalerate for 24 hrs. 200x

ABBREVIATIONS

PLATE NO. 5

Fig. No. 1

OC:Oocyte,FW:Follicular wall, CT:Connective tissue,NU:Nucleolus, AF:Atretic follicle.

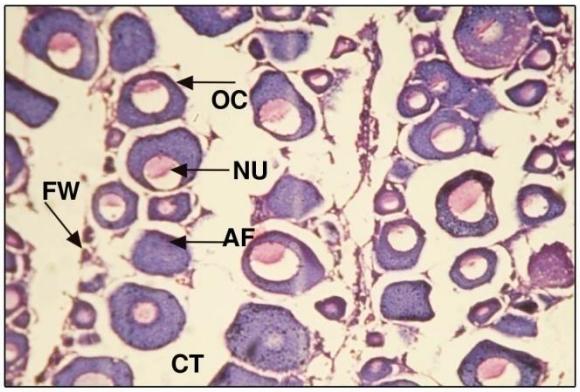
Fig. No. 2

OC:Oocyte,FW:Follicular wall, NU:Nucleolus, AF:Atretic follicle .

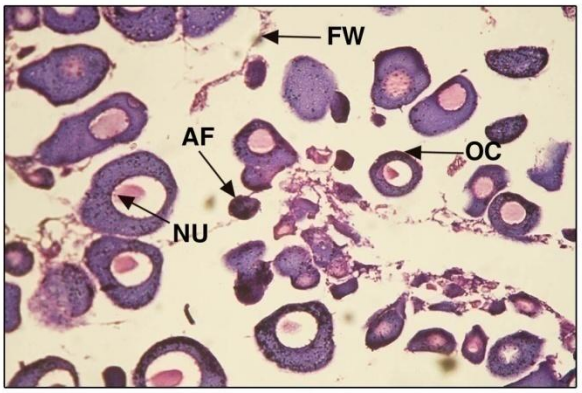
Fig. No. 3

OC:Oocyte, FW:Follicular wall, CT:Connective tissue, NU:Nucleolus, AF:Atretic follicle.

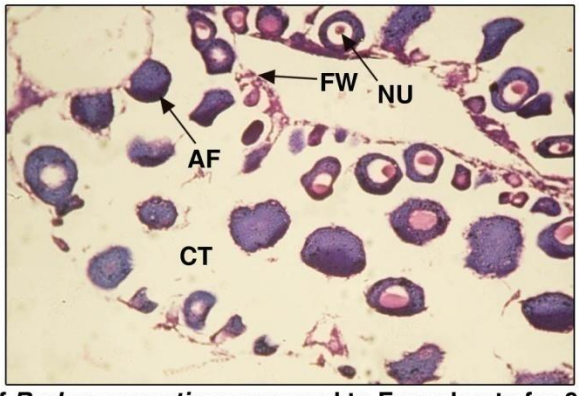
PLATE - 5



T.S. of Ovary of *Barbus carnaticus* exposed to Fenvalerate for 48 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Fenvalerate for 72 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Fenvalerate for 96 hrs. 200x

ABBREVIATIONS

PLATE NO. 6

Fig. No. 1

PS:Primary spermatocytes, SS: Secondary spermatocytes, ST: Spermatids, SG: Spermatogonia, SP:Sperms.

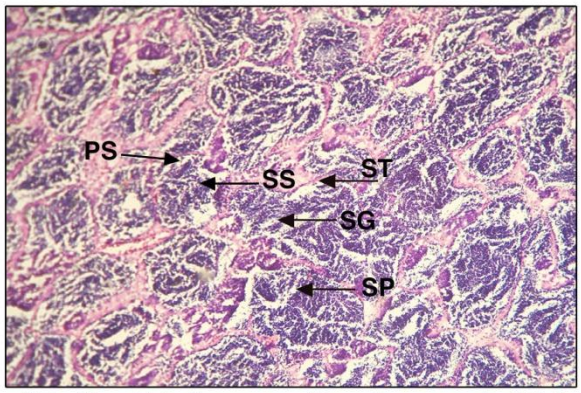
Fig. No. 2

SS: Secondary spermatocytes, ST: Spermatids, SG: Spermatogonia, SP:Sperms.

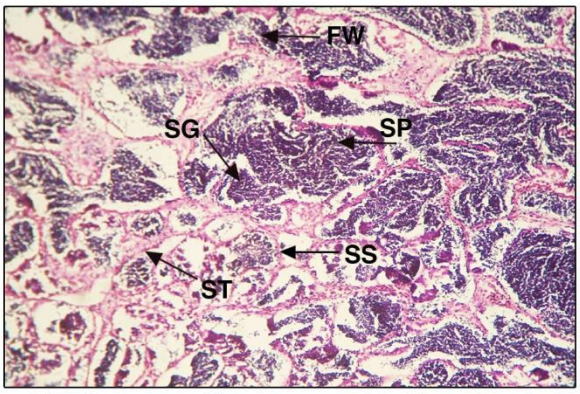
Fig. No. 3

SS: Secondary spermatocytes, ST: Spermatids, SG: Spermatogonia, SP:Sperms.

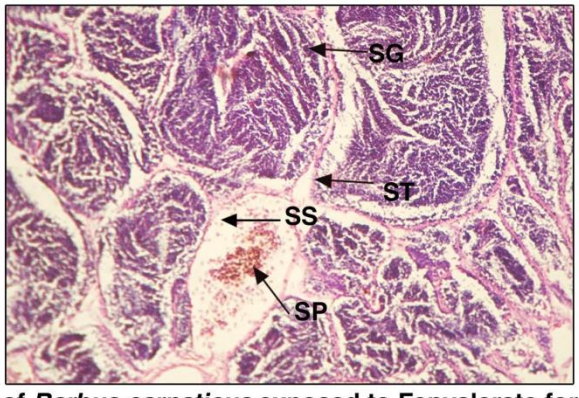
PLATE - 6



T.S. of Testis of *Barbus carnaticus* from control fish 200x



T.S. of Testis of *Barbus carnaticus* exposed to Fenvalerate for 24 hrs. 200x



T.S. of Testis of *Barbus carnaticus* exposed to Fenvalerate for 48 hrs. 200x

ABBREVIATIONS

PLATE NO. 7

Fig. No. 1

SP:Sperms, ST: Spermatids, SG: Spermatogonium.

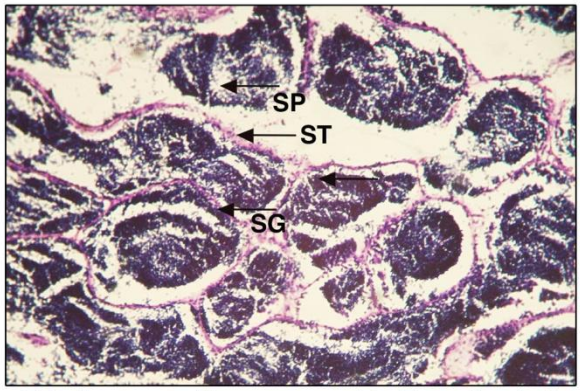
Fig. No. 2

SP:Sperms, ST: Spermatids, SG: Spermatogonium, SS:Secondary spermatocytes.

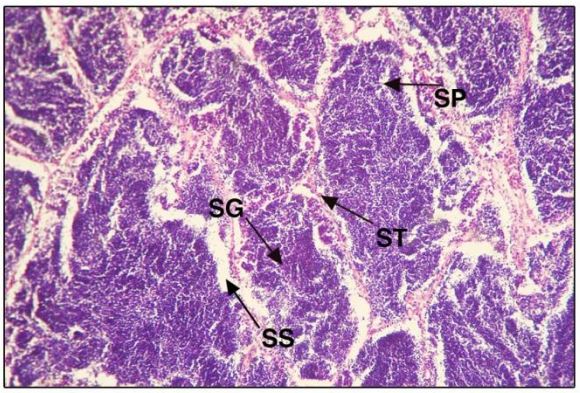
Fig. No. 3

HC : Hepatocyte, S : Sinus.

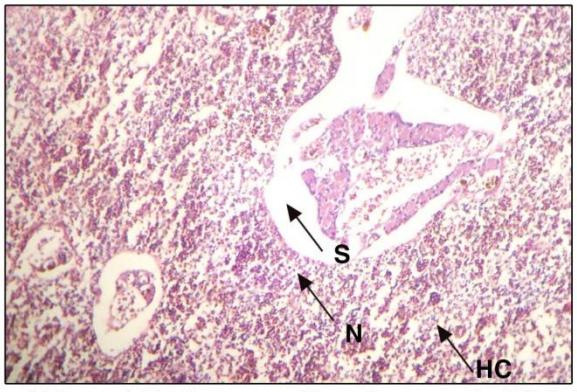
PLATE - 7



T.S. of Testis of *Barbus carnaticus* exposed to Fenvalerate for 72 hrs. 200x



T.S. of Testis of *Barbus carnaticus* exposed to Fenvalerate for 96 hrs. 200x



T.S. of Liver of *Barbus carnaticus* from control fish 200x

ABBREVIATIONS

PLATE NO. 8

Fig. No. 1

HC : Hepatocyte, S : Sinus, N : Nucleus, HE:Haemorhage.

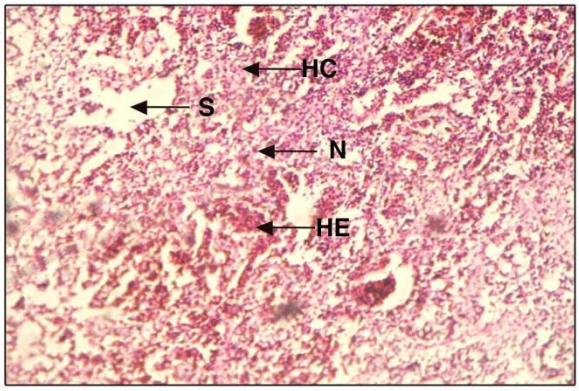
Fig. No. 2

HC : Hepatocyte, S : Sinus, N : Nucleus, HE : Haemorhage

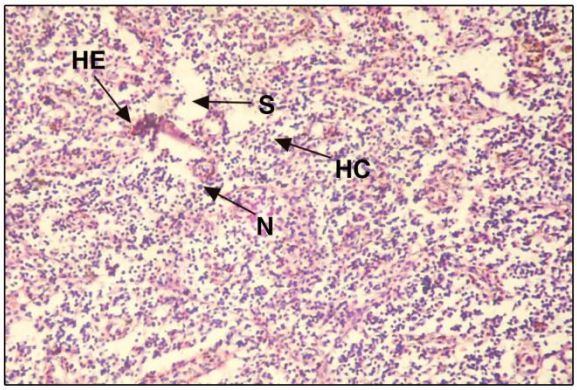
Fig. No. 3

HC : Hepatocyte, N : Nucleus, HE : Hemorage, VC:Vacuolization, NC:Necrosis.

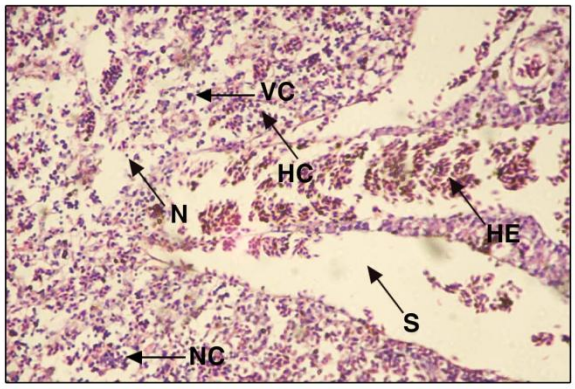
PLATE - 8



T.S. of Liver of *Barbus carnaticus* exposed to Atrazine for 24 hrs. 40x



T.S. of Liver of *Barbus carnaticus* exposed to Atrazine for 48 hrs. 40x



T.S. of Liver of *Barbus carnaticus* exposed to Atrazine for 72 hrs. 40x

ABBREVIATIONS

PLATE NO. 9

Fig. No. 1

HC : Hepatocyte, HE:Haemorhage, S : Sinus, N : Nucleus

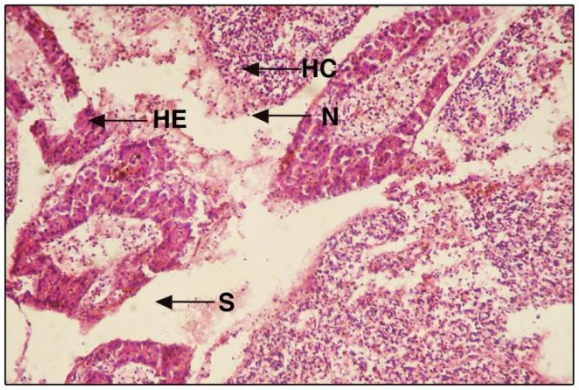
Fig. No. 2

GL: Glomerulus, UT: Uriniferous tubule, CT: Connective tissue ,S: Sinus.

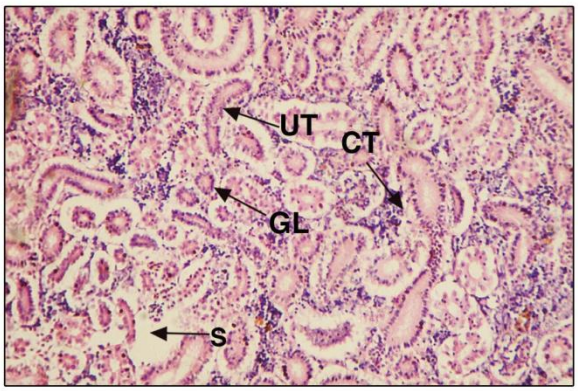
Fig. No. 3

GL: Glomerulus, UT: Uriniferous tubule, CT: Connective tissue , S: Sinus .

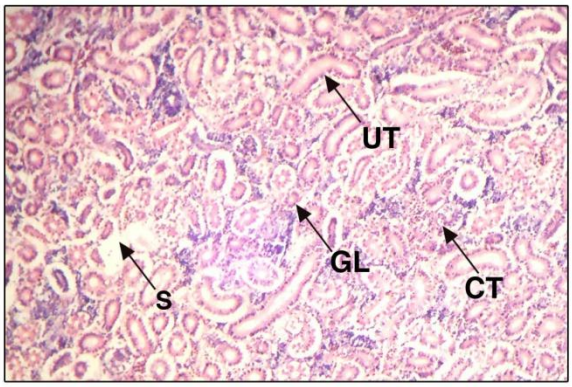
PLATE - 9



T.S. of Liver of *Barbus carnaticus* exposed to Atrazine for 96 hrs. 200x



T.S. of Kidney from *Barbus carnaticus* control 200x



T.S. of Kidney of *Barbus carnaticus* exposed to Atrazine for 24 hrs. 200x

ABBREVIATIONS

PLATE NO. 10

Fig. No. 1

GL:Glomerulus, UT: Uriniferous tubule, S: Sinus.

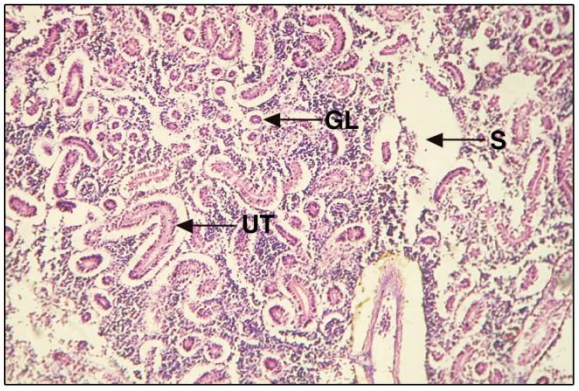
Fig. No. 2

GL:Glomerulus, UT: Uriniferous tubule, CT:Connective tissue, S : Sinus.

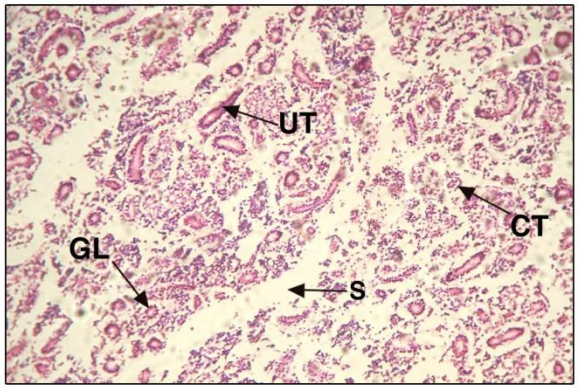
Fig. No. 3

GL:Glomerulus, UT: Uriniferous tubule, CT:Connective tissue, S : Sinus .

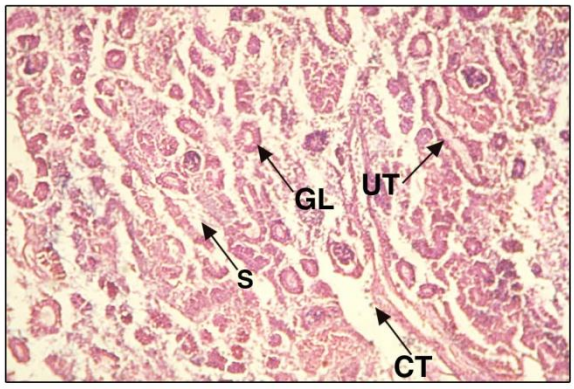
PLATE - 10



T.S. of Kidney of *Barbus carnaticus* exposed to Atrazine for 48 hrs. 200x



T.S. of Kidney of *Barbus carnaticus* exposed to Atrazine for 72 hrs. 200x



T.S. of Kidney of *Barbus carnaticus* exposed to Atrazine for 96 hrs. 200x

ABBREVIATIONS

PLATE NO. 11

Fig. No. 1

OC:Oocyte, OW: Ovarian wall , N: Nucleus, NU: Nucleous.

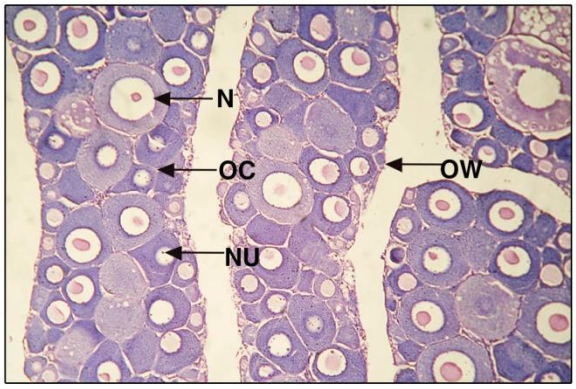
Fig. No. 2

OC:Oocyte, OW: Ovarian wall , N: Nucleus, NU: Nucleous , CT:Connective tissue, AF: Atretic follicle.

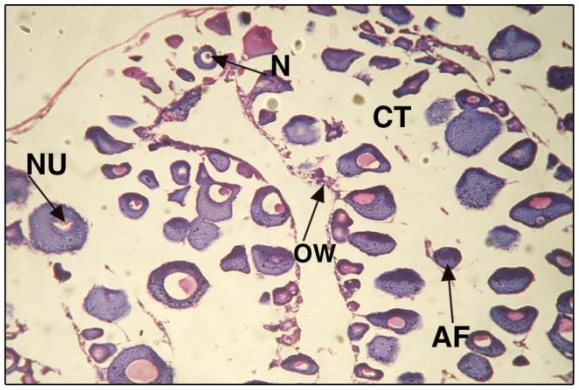
Fig. No. 3

OW: Ovarian wall , N: Nucleus, NU: Nucleous , CT:Connective tissue, AF: Atretic follicle.

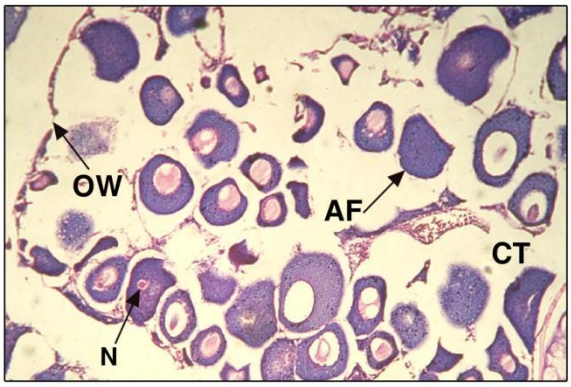
PLATE - 11



T.S. of Ovary of *Barbus carnaticus* control 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Atrazine for 24 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Atrazine for 48 hrs. 200x

ABBREVIATIONS

PLATE NO. 12

Fig. No. 1

OW:Ovarian wall, CT: Connective tissue, OC: Oocyte, AF: Atretic follicle, N : Nucleus, NU:Nucleous.

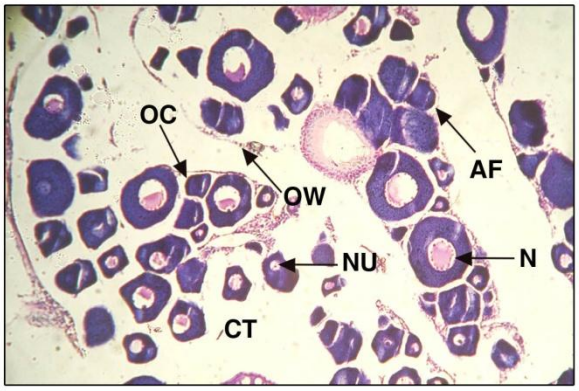
Fig. No. 2

OW:Ovarian wall, CT: Connective tissue, AF: Atretic follicle, N : Nucleus, NU:Nucleous.

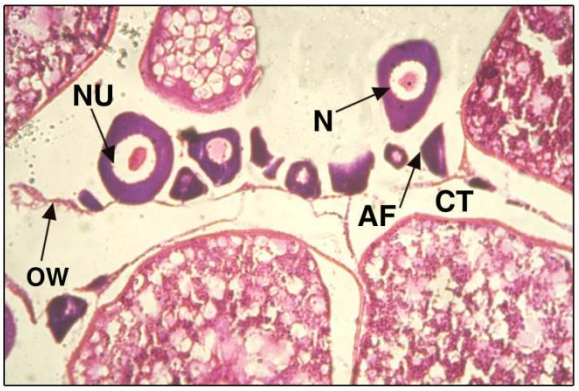
Fig. No. 3

ST:Spermatids, SG: Spermatogonia, SP: Sperms.

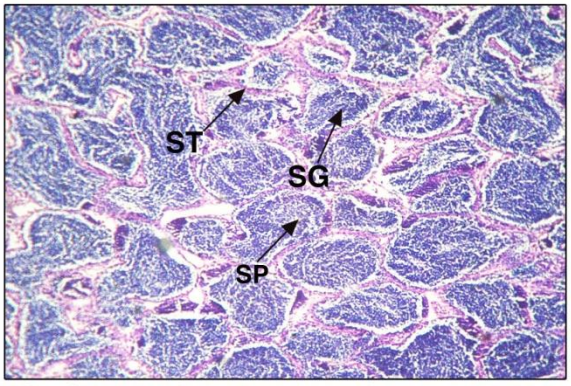
PLATE - 12



T.S. of Ovary of *Barbus carnaticus* exposed to Atrazine for 72 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Atrazine for 96 hrs. 200x



T.S. of Testis of *Barbus carnaticus* control 200x

ABBREVIATIONS

PLATE NO. 13

Fig. No. 1

SP: Sperms, SG: Spermatogonia, ST: Spermatids.

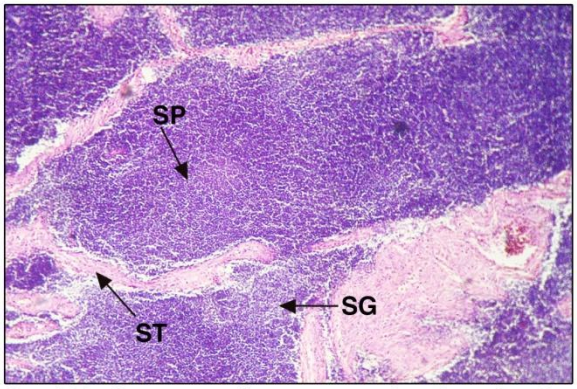
Fig. No. 2

ST: Spermatids, SG: Spermatogonia , SP:Sperms, S:Sinus.

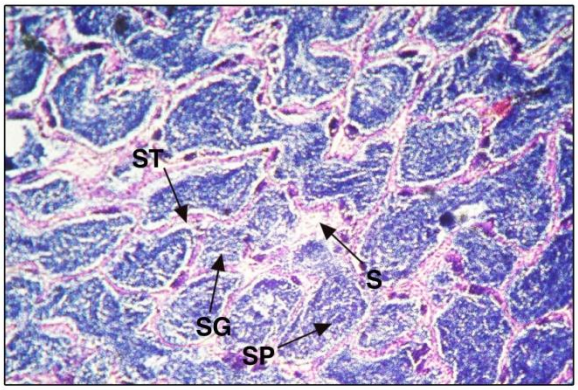
Fig. No. 3

ST: Spermatids, SG: Spermatogonia , SP:Sperms, S:Sinus.

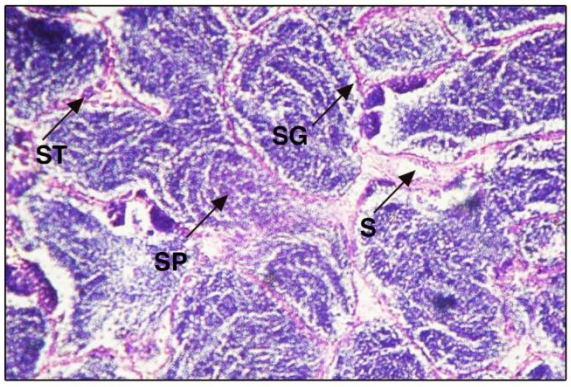
PLATE - 13



T.S. of Testis of *Barbus carnaticus* exposed to Atrazine for 24 hrs. 200x



T.S. of Testis of *Barbus carnaticus* exposed to Atrazine for 48 hrs. 200x



T.S. of Testis of *Barbus carnaticus* exposed to Atrazine for 72 hrs. 200x

ABBREVIATIONS

PLATE NO. 14

Fig. No. 1

SP:Sperms, SG:Spermatogonia, ST:Spermatids, S : Sinus.

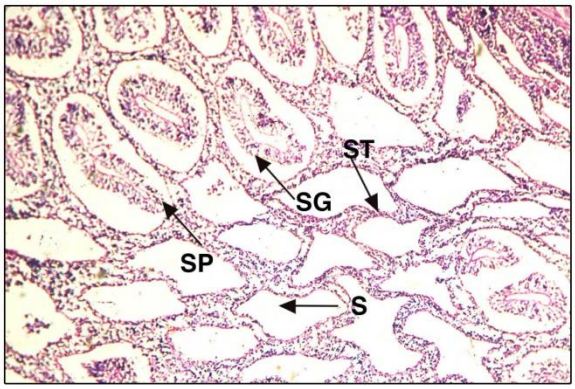
Fig. No. 2

S : Sinus, N : Nucleus, HC : Hepatocyte

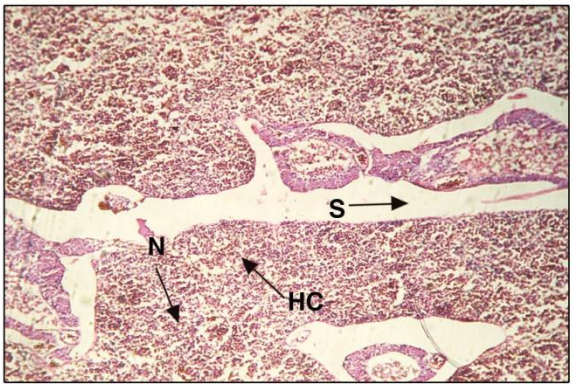
Fig. No. 3

HC : Hepatocyte, S : Sinus, N : Nucleus, NE : Necrosis.

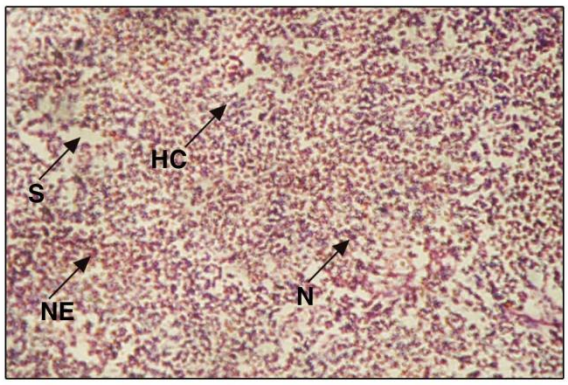
PLATE - 14



T.S. of Testis of *Barbus carnaticus* exposed to Atrazine for 96 hrs. 200x



T.S. of Liver of *Barbus carnaticus* control 200x



T.S. of Liver of *Barbus carnaticus* exposed to Contaf for 24 hrs. 200x

ABBREVIATIONS

PLATE NO. 15

Fig. No. 1

HC : Hepatocyte, S : Sinus, N : Nucleus, NE: Necrosis.

Fig. No. 2

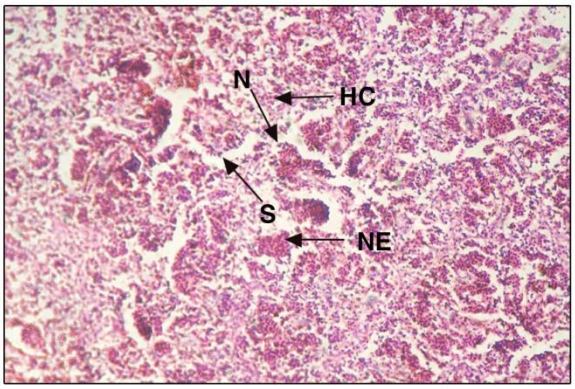
HC : Hepatocyte, S : Sinus, N : Nucleus, NE : Necrosis.

Fig. No. 3

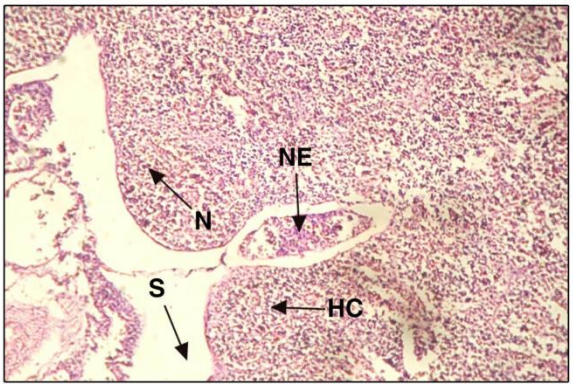
HC : Hepatocyte, S : Sinus, N : Nucleus, NE : Necrosis.

g

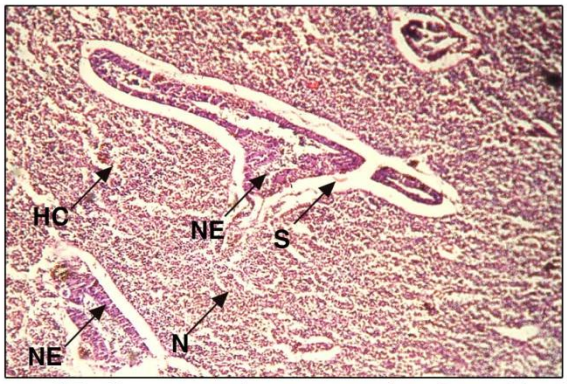
PLATE - 15



T.S. of Liver of *Barbus carnaticus* exposed to Contaf for 48 hrs. 200x



T.S. of Liver of *Barbus carnaticus* exposed to Contaf for 72 hrs. 200x



T.S. of Liver of *Barbus carnaticus* exposed to Contaf for 96 hrs. 200x

ABBREVIATIONS

PLATE NO. 16

Fig. No. 1

GL:Glomerulus, UT: Uriniferous tubule, CT:Connective tissue, NE: Necrosis, S : Sinus, N : Nucleus.

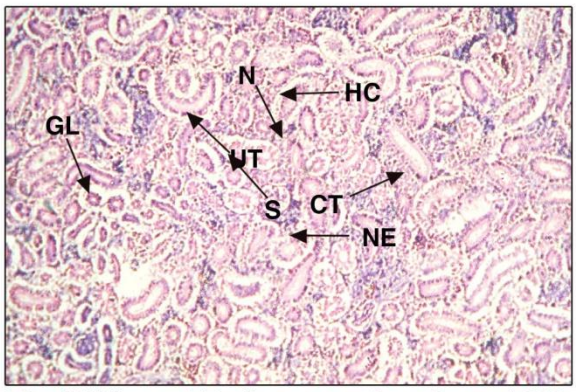
Fig. No. 2

CT: Connective tissue, UT: Uriniferous tubule , NE: Necrosis,
GL: Glomerulus, S : Sinus.

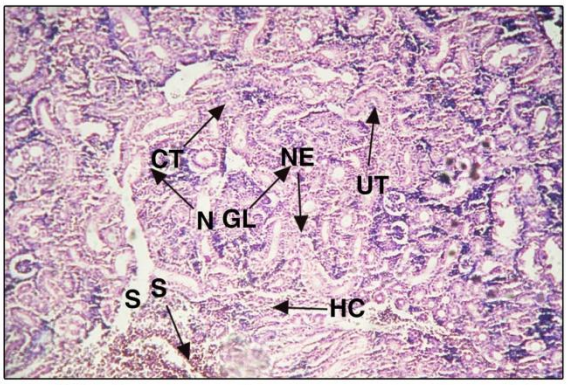
Fig. No. 3

CT: Connective tissue, UT: Uriniferous tubule GL: Glomerulus, S : Sinus.

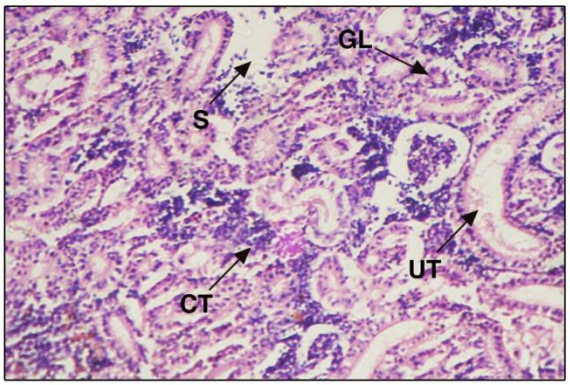
PLATE - 16



T.S. of Kidney of *Barbus carnaticus* Control 200x



T.S. of Kidney of *Barbus carnaticus* exposed to Contaf for 24 hrs. 200x



T.S. of Kidney of *Barbus carnaticus* exposed to Contaf for 48 hrs. 200x

ABBREVIATIONS

PLATE NO. 17

Fig. No. 1

CT: Connective tissue, S : Sinus, GL: Glomerulus, UT: Uriniferous tubule.

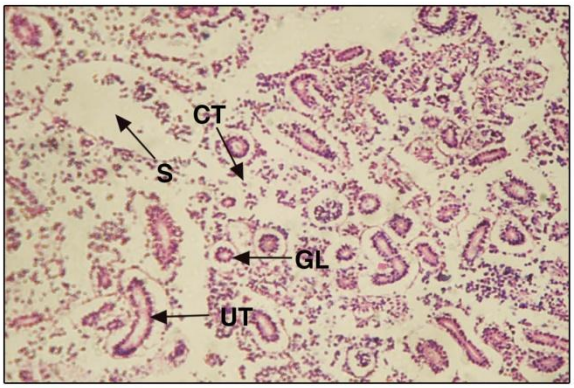
Fig. No. 2

GL:Glomerulus, UT: Uriniferous tubule, S : Sinus, CT: Connective tissue.

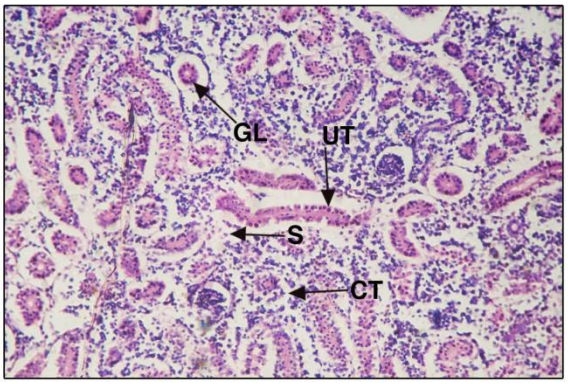
Fig. No. 3

OC:Oocyte, N : Nucleus, OW: Ovarian wall , NU: Nucleolus.

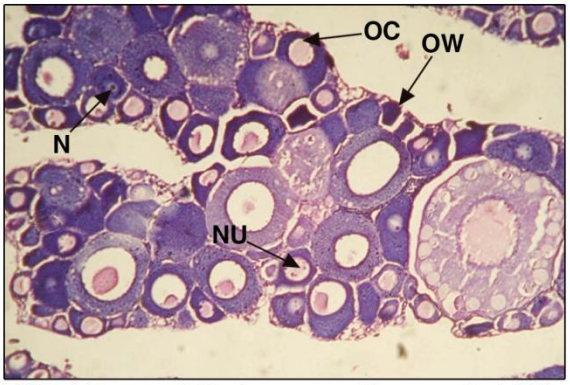
PLATE - 17



T.S. of Kidney of *Barbus carnaticus* exposed to Contaf for 72 hrs. 200x



T.S. of Kidney of *Barbus carnaticus* exposed to Contaf for 96 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* control 200x

ABBREVIATIONS

PLATE NO. 18

Fig. No. 1

NU: Nucleolus, N:Nucleus, CT: Connective tissue, AF:Atretic follicle, OW: Ovarian wall.

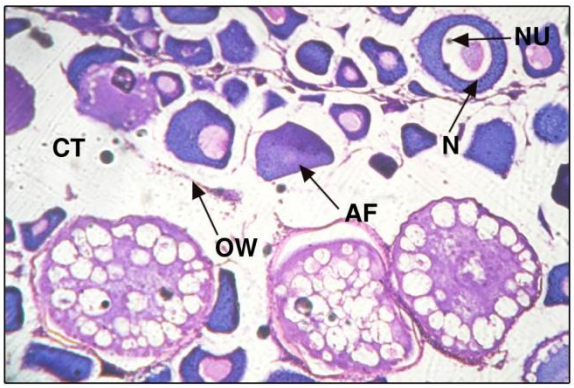
Fig. No. 2

N:Nucleus, CT: Connective tissue, AF:Atretic follicle, OW: Ovarian wall.

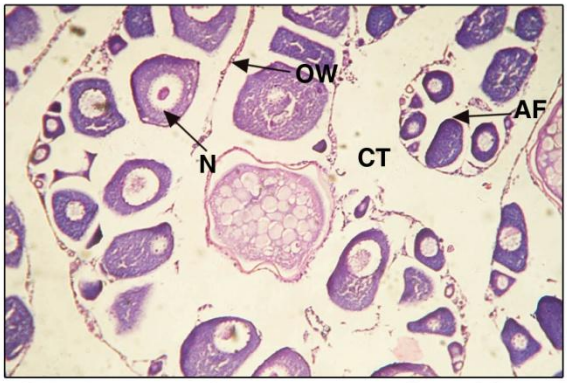
Fig. No. 3

N:Nucleus, NU:Nucleolus, , AF:Atretic follicle, OW: Ovarian wall , OC:Oocyte.

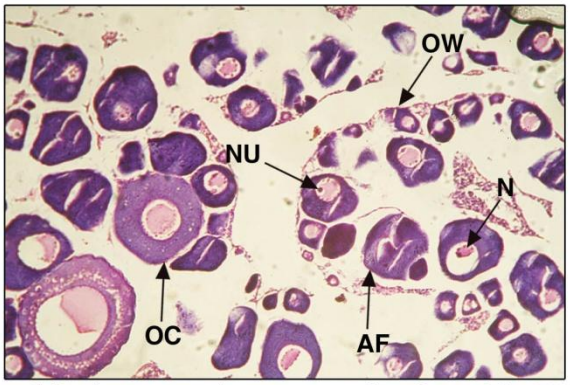
PLATE - 18



T.S. of Ovary of *Barbus carnaticus* exposed to Contaf for 24 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Contaf for 48 hrs. 200x



T.S. of Ovary of *Barbus carnaticus* exposed to Contaf for 72 hrs. 200x

ABBREVIATIONS

PLATE NO. 19

Fig. No. 1

OW:Ovarian wall, CT:Connective tissue, NU:Nucleolus, AF:Atretic follicle, N : Nucleus

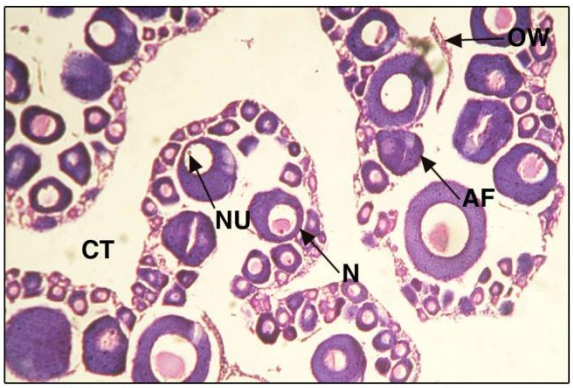
Fig. No. 2

ST: Spermatids, SP: Sperms, SG: Spermatogonia.

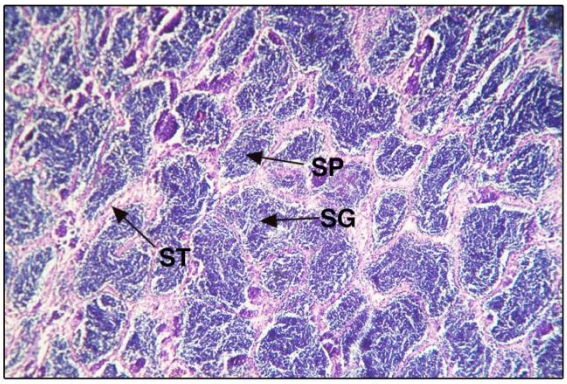
Fig. No. 3

ST: Spermatids, SP: Sperms, SG: Spermatogonia, S:Sinus.

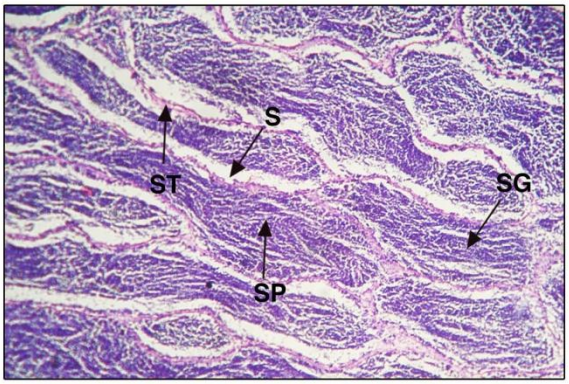
PLATE - 19



T.S. of Ovary of *Barbus carnaticus* exposed to Contaf for 96 hrs. 200x



T.S. of Testis of *Barbus carnaticus* control 200x



T.S. of Testis of *Barbus carnaticus* exposed to Contaf for 24 hrs. 200x

ABBREVIATIONS

PLATE NO. 20

Fig. No. 1

ST: Spermatids,SG: Spermatogonia, CT: Connective tissue,SP: Sperms.

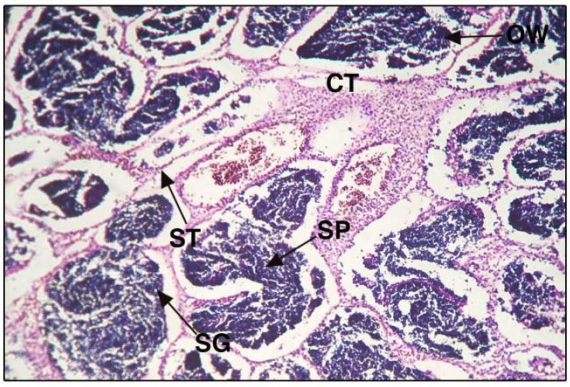
Fig. No. 2

ST: Spermatids, SP: Sperms, SG: Spermatogonia, CT: Connective tissue.

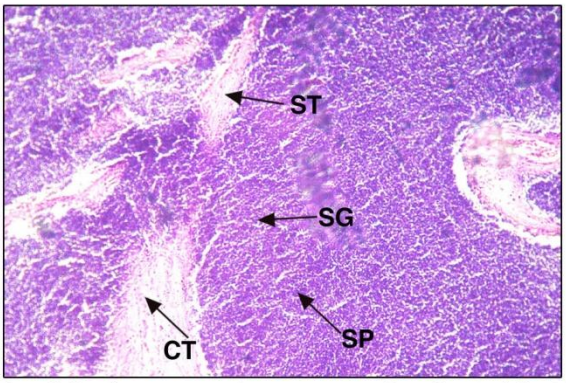
Fig. No. 3

ST: Spermatids, S: Sinus, CT: Connective tissue, SP: Sperms.

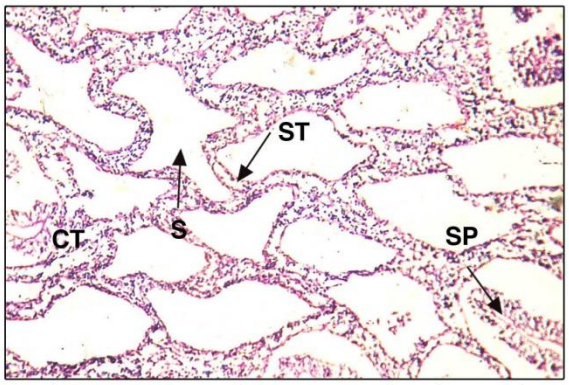
PLATE - 20



T.S. of Testis of *Barbus carnaticus* exposed to Contaf for 48 hrs. 200x



T.S. of Testis of *Barbus carnaticus* exposed to Contaf for 72 hrs. 200x



T.S. of Testis of *Barbus carnaticus* exposed to Contaf for 96 hrs. 200x

DISCUSSION

The fish liver appears, as does the liver of other vertebrates, as a key organ which controls many life functions and play a prominent role in fish physiology, both in the anabolism (proteins, lipids and carbohydrates) and catabolism (nitrogen, glycogenolysis, detoxification). It also plays an important role in vitellogenesis and when compared with mammals only a minor role in carbohydrate metabolism. The fish liver must be considered as a target organ for many biological and environmental parameters. Liver is very important organ to study the interactions between environmental factors and hepatic structures and functions.

As the liver is involved in detoxification of pollutants, it merits analysis Cassilas et al., (1983) observed cellular coagulation, necrosis, increased cytoplasmic eosinophilia and subscapular necrosis foci in English sole Parophrys vetulus injected with 3.0 ml. of carbon tetrachlonde. Datta et al., (1993 b) found changes in the diameter, cellular coagulation, and necrosis in the hepatocytes of H. fossilis exposed to malathion.

Liver being the main organ of various key metabolic pathways, the effects of a chemical usually appear primarily in the liver. Histopathological lesions caused by pesticides and industrial pollutants have been amply reported. Chemically induced liver injury resulting from chronic exposure can produce changes in liver structure with the degenerative and proliferative changes. Malathion (Dubale and shah,1979, Observrd toxic effect of malathion on liver of C.pnuctatus.Areechon and Plumb,1990,Observed toxic effect of malathion on cat fish I.punctatus. Datta et al ;1993 b also observed toxic

effect of malathion on cat fish H.fossilis.), Sumithion in C.batrachus.(Mandal and Kulshrestha 1980)

The fresh water teleost fish, B. carnaticus is an edible fish and is economically important. The present study was undertaken to evaluate the toxic effects of an Atrazine ,Contaf and Fenvalerate.

The chemical insecticide affects all the body parts of the exposed fishes. When chemical enters the body, the animal metabolizes it, so that it is delivered outside the body. This is the method of detoxification of toxic material that enters in the body. Animals use other ways of detoxification, in case where a pesticide could not be metabolized. The pesticide is scattered in different systems, consequently in different organs and tissues, in order to reduce its level of toxicity. All animals have some capacity to tolerate sub-optimal conditions. The maximum limits of tolerance achieved at extreme conditions of stress show physiologically defined boundaries, which are reflected very strongly in the structural architecture of the tissue and thereby organ, which make up the entire body of an animal. Some animals are susceptible to one pesticide while resistant to the other. The disarrangement and alterations observed in histological architecture in the various organs and tissue appears to be tissue specific, depending on time and conditions created by various environmental parameters. Many workers have reported the toxicity induced by insecticides on various organs of fish (Ishihara and Tamura, 1967, Bhattacharya and Mukherjee ,1975, Dubale and Shah 1979, Dubale and Awasthi,1982). In the present investigation histopathological effects were seen in the liver, kidney, testis, ovary etc. of freshwater fish B.carnaticus when exposed to sub lethal doses of Atrazine, Contaf and Fenvalerate for 24, 48, 72 and 96 hours

respectively. It is observed that the effect is much more fatal for 96 hours duration. It is concentration and duration dependent process.

The tissues of liver and kidney got affected and showed changes in the cytoarchitecture due to stress of organophosphate Atrazine and organochloride Contaf and pyrethroid Fenvalerate respectively. The changes indicated disruption of hepatocytes and hepatic cords and failure of physiological functions. The liver and kidney are the main target organs of the toxicants; therefore they are very sensitive. The initial histological reaction can lead to pathological changes and continuous, impairment progresses in the direction of significant decrease, which ultimately can lead to death of animal.

In the present study, the most conspicuous changes are liver cord disarray, connective tissue damage and degeneration of hepatocytes. The cytoplasm of the hepatocytes is highly degenerated and the hepatocytes structure is altered. The necrotic changes were followed by vacuolation. Liver histopathological lesions appear to be the chief changes. Severe histopathological changes such as vacuolation, necrosis, degeneration of hepatic cords with hepatic cells and lymphocytic infiltration.

Liver and kidney being the worst affected organs by toxicants. Histopathological lesions have been observed by Anees ,(1975) in the kidney of Channa punctatus. Shah ,(1980) has reported some severe changes in the kidney of Channa punctatus exposed to Malathion. Experimental liver of B. carnaticus exposed to sub lethal dose of Atrazine for 96 hours (Plate 9,Fig 1) showed small vacuoles, degenerations of hepatocytes and necrosis of cells, proliferations of ducted cells. These results are in agreement with these of Saxena et al., (1989). They reported that Malathion is more toxic than carboryl and the denova synthesis of lipid and protein in the liver of C.punctatus.

King, (1962) observed the various histopathological changes in the liver, intestine and kidney particularly cell vacuolation in guppies and brown trout fry due to D. D. T. Halper et.al., (1962) also observed hepatomas in rainbow trout due to high dosage of D.D.T. Sakar and Jamal Al lail (2005) observed Fenvalerate induced histopathological changes in the liver of the catfish Clarias gariepimus. They noticed vacuolization of the hepatocytes, blood vessel congestion, inflammatory leucocytic infiltration, necrosis and fatty infiltrations. Butchiram et.al.,(2009).Observed the tissue damages like degenerations of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels and disposition of hepatic cords observed in the liver of fish Channa punctatus (Bloch) exposed to Alachlor. In the lethal concentrations of cypermethrin the kidney C.mrigala showed reduction in renal cell number in the proximal and distal collecting tubules, which have resulted in narrowness of lumen (Prashanth M.S. 2011).

According to, Rouiller, (1964) fatty accumulation is the characteristic feature of liver damage. Andrews et.al., (1966) observed degeneration liver lesions due to heptachlor in blue gills. Widespread use of insecticides, fungicides etc. are a source of pollution in water, which has escaped from the fields. It is known that organochlorine insecticides caused histopathological changes in the liver,kidney, gill and pancreas of fishes (Bhattacharya and Mujkherjee,1975; Amminikutty and Rege, (1977). Konar,(1969) reported histopathological changes in liver and kidney of Labeo rohita with heptachlor poisoning. Copper exposure to fish organs has shown damaging effects on liver (Gardner and Laroche, 1973). According to Patil (1986) different pesticides also show effect on the physiology and endocrinology of army worm Mythimna (Pseudolata) separata.

Konar, (1969) observed swollen and vacuolated parenchymal cells in liver of carp and catfishes, due to effects of some organo- phosphorous pesticides. Influence of thiodon and agallol- 3 on the liver of Teragymnocormbus ternetzi (Boulenger) was observed by Amminikutty and Rege, (1977).The histopathological lesions induced by Malathion in the liver of Channa punctutus were studied by Dubale and Shah, (1979), and it was seen to induce hepatopathy. Malathion treated Channa punctatus showed precipitation of protoplasmic elements, spoilage of nuclei and vacuolization in the hepatic cells Shah, (1980).

Saravanan et.al.,(2010)also noticed the significant histopathological alterations in liver and gill of endosulfan treated fish Labeo rohita. Vasait ,(2002) reported most conspicuous changes in liver and kidney after exposure of Nemacheilus botia to monocrotophos. These changes are liver cord disarray, connective tissue damage and degenerations of hepatocytes. He also reported histopathological changes in kidney which are characterized by changes in the cellular nature of glomeruli and degeneration of tubular cells with vacuolation. Rashatwar and Ilyas, (1984) reported alterations in the liver such as vacuolated hepatocytes and necrosis of Nemacheilus denisoni when exposed to phosphomidon. Khillare and Wagh, (1989) observed the effect of pesticide endosulfon and Malathion on fresh water fish, Puntius stigma. He has reported necrosis, degeneration of parenchymatous cells, and vacuolation in hepatic cells, pyknosis and distortion in islets' of Langerhans and disturbed cords in liver of Puntius stigma.

Patil, (1987) observed histopathological alterations in liver of fish, Boleopthalmus dussumieri due to organophosphorous insecticide, Monocrotophos. He has observed necrosis, vacuolation and fatty infiltration. Palanichamy and Karpagaganpath, (1987) observed the

histopathological changes in gill, liver and testis of Channa punctatus exposed to Monocrotophos.

Anitha, et.al., (1997) observed the histopathological changes in the liver of Channa punctatus induced by aquatic pollutants. The vacuolation of hepatocytes , pyknosis in many of the necrotic cells and necrosis of exocrine pancreatic tissue and disintegration of the sinusoids reported in liver of Channa punctatus (Bloch) induced by chronic nonlethal levels of elsan, mercury and ammonia. Grinwis et al;(2000) observed the (pre) neoplastic; liver lesion and lymphocytic viral disease in European flounder (Platichthys flesus) due to 2,3,7, 8, -tetrachlorodiben 20-p-aioxin (TCDD) Tilak, et.al., (2001b) observed the tissue damages like necrosis and vacuolar degeneration in fish, Ctenophoryngodon idella (Valenciennes) when exposed to technical and sublthal concentration of 20% EC fenvalerate a synthetic pyrethroid. Tripathi et.al,(2001) observed the histopathological changes in Funambulus permati induced by fenvalerate. They reported loosening and enlargement of hepatocytes of under toxicity. They observed major changes with hepatic lesions with necrosis, pyknotic nuclei, vacuolation, damaged blood vessels and accumulation of cytoplasmic granules in liver.

The cytoplasm of the hepatocytes is highly degenerated and the hepatocyte structure is altered. The necrotic changes were followed by vacuolation. Liver histopathological lesions appear to be the chief changes. Severe histopathological changes such as vacuolation, necrosis, degeneration of hepatic cords with hepatic cells and lymphocytic infiltration.

Hepatic lesions in the liver tissues of fishes exposed to dichlorvos were studied by Velmurugan et. al.,(2009).They reported characterized cloudy swelling of hepatocytes, congestion, vacuolar degeneration,

karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy in the liver of Cirrhinus mrigala. Korkmaz et.al., (2009) studied the cypermethrin induced histopathological and biochemical changes in Nile tilapia (Oreochromis niloticus). They reported histopathological lesions in the gill, liver and kidney. Annes, (1975) observed the slow damage of excretory and haemopoietic units of the kidney in C.punctatus when exposed to organophosphorous insecticide.

During the present investigation, changes were observed in the kidney of B.carnaticus exposed to different concentrations of Atrazine. Histopathological damage was found in the glomerulus, renal tubules and haemopoietic tissue. A gradual increase in the damage was noticed and the severe histological lesions caused by physiological and biochemical disturbances in fish.

Mandal and Kulshrestha, (1980) observed the nephropathy in Clarias batrachus induced by 1 ppm of sumithion. The changes observed in the kidney of the treated fish include vacuolation of epithelial cells of uriniferous tubules and degenerations of glomeruli.

Rashatwar and Ilyas, (1984) reported shrinkage of glomeruli and swelling of the renal tubules in kidney of Nemacheilus denisoni, when exposed to phosphomidon. Mohapatra and Noble, (1992) observed the effect of organophosphate pesticide, Nuvan on fish Liza persia. They observed enlargement of renal tubules, necrosis of epithelial tubular cells in kidney. Saxena, (1991) has worked on the histopathological changes in the kidney of Channa punctatus induced by rogor. She observed clumping of nuclei of epithelial cells of renal tubules, shrinkage and degeneration of glomeruli and necrosis of haemopoietic tissue.

The kidney of B.carnaticus exposed to sublethal dose of Fenvalerate showed damaged proximal and distal tubule and sinus

appeared in connective tissue, necrosis, swelling in renal tubules (Plate-4,Fig.1) As toxic products are eliminated through the kidney, it is susceptible to sublethal doses of Fenvalerate. It might have caused degenerated changes in renal tubules and glomerulus i.e. necrosis in the proximal tubules and glomerulus of kidney. Degenerative changes in epithelial cells of collecting tubules of Tilapia mossambica exposed to heptachlor has been reported by Radhaiah,(1985) shrinkage of glomerulus, were reported in Nemachelius denisoni (Day) exposed to phosphamidan (Rashatwar and Ilyas 1984). Similar results on fresh water teleosts are reported by Koteswara rao,(2003) and Tilak et al., (2004). Anitha et al; (1995) reported the degeneration of uriniferous tubules indicating impairment of normal functioning of kidney in Heteropneustes fossilis due to industrial effluents. Anitha et al; (1997) reported severe histopathological damage in kidney of Channa punctatus due to aquatic pollutants. Grinvis et al; (2000) observed the strong immune reactivity in a distinct cell population of the hematopoietic tissue in kidney of European flounder Platichthys flesus due to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD).

In the present study, histopathological changes in kidney are characterized by changes in the cellular nature of glomeruli and degeneration of tubular cells with vacuolation. At the increased duration from 24hours to 96 hours there was significant degeneration of renal tubules and heavy lymphocytic infiltration. The shrunken glomeruli change in position of nuclei in epithelial cells with pyknosis observed in tissue frequently. Necrosis was also observed. The renal tubules were observed in different stages of progressive degeneration. Severe vacuolar degeneration of the tubular cells, with a gradual degeneration of tubules suggest impairment in reabsorption of electrolytes in to the circulatory systems. It will affect urinary filtration process leading to an imbalance in

osmotic regulation of body fluids and affects pH which influences adversely various enzymatic processes in the organism as the enzyme mediated biochemical process are extremely sensitive to alterations in electrolyte composition and pH.

As toxic products are eliminated through the kidney, it is susceptible to sub lethal doses of Fenvalerate. It might have caused degenerative changes in renal tubules and glomerulus i.e. necrosis in the proximal tubules and glomerules of kidney. Degenerative changes in epithelial cells of collecting tubules of Tilapia mossambica exposed to heptachlor has been reported by Radhaiah ,(1985) shrinkage of glomerulus, were reported in Nemachelius denisoni (Day) exposed to phosphamidan (Rashatwar & Ilyas 1984) The vacuolation was Similar results on freshwater teleosts are reported by Kotesware rao (2003) and Tilak et al. (2004).

Degenerative changes in epithelial cells of proximal tubules and haemopoietic tissues, severe necrosis in the proximal tubules leading to the formation of vacuoles. When fish is happened to expose to pesticides, they cause irrepairable architectural changes in the vital organs like kidney making the fish less fit for better survival. These histological changes can alter various physiological activities of fish such as release of various enzymes and consequently metabolism is affected.

Ovary: The present investigation was undertaken to study the histopathological changes occurring in the gonads after exposure to sublethal dose of Fenvalerate , Atrazine and Contaf respectively.

Follicular cells are desrupted. Nucleolus shows condensation of crescent shaped dark granules at one side. Degeneration of epithelial cells causes vacuolation, breakdown of germinal vesical, many disrupted oogonia are the changes caused due to the exposure of ovary of B.

carnaticus to sublethal dose of Fenvalerate. Similarly the destroyed and disrupted follicular epithelial cells. Nucleolus showed condensation of dark granules at one side, vacuolation at periphery of oocyte are the changes caused by sublethal dose of Atrazine. It also shows degeneration of oocytes and atresiaand destruction of maturing yolky oocytes because of which ovary gets reduced in structure. Inhibition of growth of oocytes, especially the atretic follicles was observed when exposed to sublethal dose of Contaf.All these changes are similar to following investigations.

Chronic exposure to endrin (Chlorinated hydrocarbon) is known to result in the formationof a typical Oocytes, increased follicular atresia, inhibition of ovulation in Salmo clarki, Eiler, (1981). Similarly increased follicular atresia is observed in Sarthoerodon mossambicus when exposed to malathion.Shukla and Pandey, (1984) The responses of the ovary to different biocides may also vary. In Channa punctatus treated with two different biocides, carbofuran and fenitrothion at toxicologically safe concentration, relatively more oocytes underwent atresia of follicles following 120 days exposure to fenitrothion. On the other hand about 60% atretic follicles were found in the ovaries of the carbofuran treated fish following the 120 days exposure as against 12% in controls .Mani and Saxena, (1985) disrupted follicular cells. Nucleolus shows condensation of crescent shaped dark granules at one side. In recent years fish have been exposed to increased levels of pollutants in water in which they live. Several studies have been carried out on the effect of biocides on fish under laboratory conditions. Saxena and Bhatia, (1983) Shukla and Pandey, (1984) Mani and Saxena, (1985) Lam, (1983) Patil and Saidapur, (1989)

The effects of organophosphorus and organochlorine insecticides on ovarian growth, ovulation and other aspects of reproduction in various species of teleost have been investigated (Mani and Saxena, 1985; Haider and Inbaraj, 1988)Murugesan and Hanifia, 1992, observed histopathological and histochemical changes in oocytes of the air breathing fish, Heteropneustes fossilis (Bloch) after exposure to textile mill effluent. They observed complete karyolysis, dissappearance of the chromatin reticulum of the nucleus and vacuolation of the cytoplasm. Sukamar and Karpaganapathy (1992) found fewer mature oocytes, most of which had become atreatic in the ovaries of fish, exposed to sublethal concentrations of carbofuran.

The histological abnormalities in ovaries may be caused by several factors viz. ionizing radiations, electric current, parasitic infections, xenobiotic toxicants (Sarojini and Victor, 1985) and by a variety of effluents and aquatic pollutants (Shukla et al., 1984, Saxena and Garg, 1978, Johnson et al., 1998, Mc Comic et al., 1989, Kumar et al., 2000). Almost similar histological findings were reported by Hossain et al., (2002) in the ovaries of Anabas testudineus.

Ramchandran Mohan (2000) under sublethal exposure of Malathion significant reduction in the ovarian weight and diameter of developing oocytes and also degeneration of growing oocytes and reabsorption of yolk of oocytes exhibited atresia in Glassogobius giuris. Fishes regress the gonadal development to a great extent and thus the pesticides have been considered as important inhibitors of gonadal activity Donaldson, 1975; Lam,1983;; Kime 1995. Decrease in gonadosomal index seems to be the most important and common effect in female fish due to the exposure to pesticides .Choudhary et al;,(1993) the increase in the follicular atresia of fish Anabas testudineus was next

most obvious influence of pesticides on fish ovary. The gonadosomatic index, maximum oocyte diameter and nuclear diamerer were reduced due to retarded development of oocytes while the numbers of atretic follicles were increased further. Impact of these pesticides was much greater at 96 hours. The findings have been correlated by the biochemical studies done on fish under influence of Nuvan and Dimecron (Saxena and Saksena, 1996). Control fish ovary shows normal distinct ovarian wall, small oocytes, follicular cells with distinct nucleus and all are in compact form arranged in ovigerous lamellae (Plate-4,Fig.2). The histology of experimental fish ovary showed disrupted follicular epithelial cells. Nucleolus showed condensation of crescent shaped dark granules at one side. Degeneration of epithelial cells causes vacuolation, breakdown of germinal vesical, many disrupted oogonia.Maximum damage is produced at 96 hours exposure of Fenvalerate, Atrazine and Contaf respectively.(Plate-5,Fig.3),(Plate-12,Fig.2),(Plate-19,Fig.1).Most of workers have shown that the fishes exposed to pesticides led toward steroidogenesis (Kapur et al., 1978). Stopage of development of advanced oocyte stages and thus reducing the number of viable oocytes (Saxena&Garg; 1978; Yasuno et al. 1980; Mani&Saxena, 1985). The increase in follicular atresia was obvious due to effect of pesticides of follicular atresia were evident in ovary of Channa orientalis exposed to Nuvan and Dimecron as have been observed (Saxena and Saksena 1996)In the case of certain fishes.(Shukla et al.; 1984,Mani & Saxena, 1985; Ghosh, 1986; Singh & Sahai, 1986; Khillare & Wagh, 1987; Patwardhan & Gaikwad , 1990; Dutta et al, 1994).

Testes: - Testis of control fish were composed of lobules showing active spermatogenesis sperm nests were found in majority of the lobules. Spermatids were the predominant, followed by dividing spermatocytes and secondary spormatogonia .Majority of the spermatogonic nests were

in active state of division, the peripheral part of lobules had a normal healthy look sertoli cells were normal with granular cytoplasm containing a large nucleus with a peripheral nucleolus in a number of places. The inter lobular septa were marked by the presence of small and large aggregations of polygonal leydig cells. These possessed large carminophilic nucles containing annular chromatin and two or more nucleoli. Their cytoplasm was faintly aniline blue positive and often contained small vacuoles.

Fish exposed to sub lethal concentrations of Atrazine for different exposure periods showed considerable degree of alteration in the histology of testes. In testes the seminiferous tubules are normally of varying shapes and sizes, each tubule has a definite thin fibrous wall which is not distinguished after spawning.The testes of B.carnaticus showed significant changes on exposure to sub lethal concentrations of Atrazine. It shows reduction in the number and condensation of spermatogonic cells as well as inflammation of cells ,contraction and vacuolation of tubules. Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants (Sokal et al., 1985; Ruby et al., 1986, 1987) Exposure of Fenvalerate is responsible for histopathological damage of fish testes. In term of vacuolization of tubular cells and distortion of seminiferous cells along with inflammatory lesions. The degenerative changes in seminiferous tubules, enlarged interstitium and hemorrhage in intertubular area in albino rats exposed to pesticides have been reported. (Dutta and Dikshith, 1973); Nigam et al; (1979) Baronia and Sahai (1993). Katti and Sathyanesan (1985) observed chronic effect of lead and cadmium on testicular structure of the catfish,Clarias batrachus. Zutshi (2005) observed the effect of fenthion on the testes of Glassogobius

giuris. They observed reduction in size with spermatids and sperms in degenerating condition.

Study on heavy metals also revealed a profound reduction in the size of the gonads and extensive destruction of the germinal elements in both sexes following chronic exposure to different concentrations of heavy met als.The mature stages were very largely destroyed while earlier stages particularly in the testis, under went extensive atrophy at the chronic concentrations.An identical arrest of spermatogenesis was also reported by Ahsan and Ahsan, (1974) in cadmium injected C.batrachus.

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