Histopathological Investigation On Superoxide Dismutase (SOD) Enzyme Activity Of Fresh Water Teleost Fish (*Labeo rohita*)

Vijeta Chaturvedi[1*], Dev Brat Mishra[2]

[1*]Research Scholar, Fish Biology, Research Lab, Department of Zoology, T.D.P.G. College, Jaunpur
[2*]Assistant Professor, Fish Biology, Research Lab, Department of Zoology, T.D.P.G. College, Jaunpur

Abstract
Present paper deals with the investigation of superoxide on the physiological impacts of environmental stressors, namely pollution, on the health of freshwater teleost fish by investigating their histopathological hepatosomatic index (HSI) and superoxide dismutase (SOD) enzyme activity. Bindeshwari Fishery pond in Bhilampur, Jaunpur district, was used to collect fish samples. The fish's liver, kidneys, testicles, and ovaries showed major changes after being exposed to the contaminants, according to histopathological analyses. Normal cellular architecture and well-organized liver structures were hallmarks of the healthy histological findings seen in the control group. Atrazine, contaf, and fenvalerate, on the other hand, caused oxidative stress symptoms in fish, such as inflammation, cellular necrosis, and structural alterations in hepatocytes, which were associated with elevated SOD activity. These results point to a physiological reaction to environmental pollutants that cause oxidative damage.

Keywords- histopathological, hepatosomatic index (HSI), teleost, SOD, inflammation

Introduction

Histopathology plays a crucial role in assessing fish health, offering a detailed and microscopic examination of tissue structure and function. By examining tissues at the cellular level, histopathology provides valuable insights into the nature and extent of diseases, infections, and environmental stressors that may affect fish populations. This method is particularly important for diagnosing various diseases caused by pathogens like bacteria, viruses, parasites, or fungi, as well as identifying toxicological impacts due to pollutants in aquatic environments.

Histopathological analysis allows researchers and veterinarians to detect early-stage infections and chronic diseases that may not be apparent through external examination or other diagnostic methods. For example, subtle cellular changes in organs such as the gills, liver, kidneys, or spleen can indicate stress, inflammation, or damage long before clinical signs become evident. By evaluating lesions, tissue degeneration, or abnormal growths, histopathologists can pinpoint the underlying causes of morbidity and mortality in fish populations.

Furthermore, this technique is essential in monitoring the health of farmed fish in aquaculture, where disease outbreaks can have devastating economic impacts. Regular histopathological assessments can help in the early detection of diseases, enabling timely interventions to prevent large-scale losses. In wild fish populations, histopathology is equally valuable, as it can reveal the effects of environmental changes, such as pollution, temperature fluctuations, or habitat destruction, on fish health.

Overall, histopathology provides a comprehensive understanding of fish health by linking visible disease signs to their cellular and tissue-level causes, making it an indispensable tool in both aquaculture management and environmental conservation efforts.

Review of Literature

A., Dr. & M., Dr. (2022). A widespread and powerful antioxidant enzyme called superoxide dismutase (SOD) is responsible for scavenging reactive oxygen species (ROS), such as superoxide radicals. It is possible that these ROS are the root cause of the DNA damage, protein damage, and lipid damage induced by oxidative stress. When free radicals (ROS) build up in the body, they contribute significantly to the development of diseases associated with aging. Limiting the production of harmful reactive oxygen species (ROS) is an important defense mechanism against a wide variety of illnesses. Living things rely on the enzyme superoxide dismutase (SOD) to protect their cells from oxidative stress. The superoxide dismutase (SOD) enzyme has the potential to aid in the fight against reactive oxygen species (ROS). Within the context of several physiological and pathological disorders, this review explores the therapeutic effects of superoxide dismutase (SOD). In order to improve the efficacy of superoxide dismutase (SOD) administration for antioxidant treatment, many immobilization enzyme systems—mounted delivery methods—have been created. For the simple reason that there may be problems when SOD or SOD mimics are applied topically.

Batool, Raufa et al., (2022). In the battle against ROS, or reactive oxygen species, the ever-present metalloenzyme superoxide dismutases (SODs) serve as the first barrier. The detoxification process relies on this enzyme because of its critical function in neutralizing physiologically generated superoxide radicals. As a result of the catalase and peroxidase enzyme components working together, superoxide radicals are able to be dismutated into H_2O_2, water, and oxygen. Plant SOD isoforms are distinct from one another due to the presence of certain metal ions in their active areas.

Adhikari, Aniket & De, Madhusnata. (2022). A higher risk of cancer, namely buccal or oral cavity cancer, has been linked to the habit of chewing betel quid (BQ), which is common in eastern and northeastern India. Also, BQ is one among the several mood-enhancing drugs utilized by Indians. Betel leaves (Piper betel) encase the contents, which also include slaked lime (calcium oxide and calcium hydroxide), areca nut (Areca catechu), catechu (Acacia catechu), and other substances. Chemicals manufactured by BQ have been classified as Group I human carcinogens by the International Agency for Research on Cancer (IARC).

Karmakar, Arnab et al., (2022). One of the most common antioxidant enzymes in aerobic organisms is superoxide dismutase, or SOD. Protecting against oxidative stress and neutralizing superoxide radicals (O2•→) are its principal roles. Different variants of this enzyme have evolved separately in prokaryotic and eukaryotic creatures.

Rispandi, Marsal et al., (2022). A more robust immune system is better able to fend off influenza virus infection when frequent moderate exercise raises the sensitivity of endogenous antioxidants. Antioxidants counteract the free radicals produced by physical activity by virtue of the phenolic chemicals they contain. To be more precise, this study makes use of a pre- and post-test experimental design. Twenty healthy, fit males (ranging in age from 20 to 22) who did not smoke and who did not take any kind of antioxidant supplement in the two weeks leading up to or during the trial were involved in the research. Researchers randomly assigned participants to one of two groups: those who engaged in regular physical activity but did not take antioxidants (group A) or those who did both (group B).

Bharti, Sandhya & Rasool, Fazle. (2021). Excess pesticide residues have found their way into the nearby aquatic environment, affecting the fish population in particular, owing to the extensive usage of these chemicals and the inadequate management of their disposal. For various periods (1, 4, 8, 12 days), biochemical and histological biomarkers of the blood and hepatorenal tissues of Channa punctatus were used to assess the effects of a low dosage of malathion (0.4 mg/L; 1/20th of 96-h LC50 value). This was done because bodies of water naturally dilute insecticides. Studying the effect that is not immediately apparent was the objective of this. Hepatorenal tissue weight, condition factor (K), hematocrit saturation index (HSI), and kappa-light chain thickness (KSI) all declined as pesticide exposure percentages rose. Fish treated to malathion for twelve days showed several metabolic changes. Some of the changes that occurred were a decrease of 72.23 percent in blood glucose levels and an increase of 29.03 percent in protein concentrations.

Reddy, P. (2021). The principal water supply for agricultural, drinking, and industrial uses in the Nagda area of Western India is the Chambal River. The research team behind this project hopes to learn two things: first, if fish health is related to water quality, and second, how rivers affect the current fish health situation. *Labeo rohita*'s HSI, condition factor, and coefficient of determination data were recorded in situ in Chambal River in the winter of 2020 as part of the present experiment.

Rosa, Arianna et al., (2021). Taking a supplement containing superoxide dismutase (SOD) may help the body's natural antioxidant mechanisms accomplish their job of removing harmful free radicals. This has several potential applications in the field of pathology. Drawing attention to both existing problems and new delivery systems being developed to solve bioavailability issues. An electronic search was performed on PubMed using the following terms: "SOD," "SOD mimetics," and "SOD supplementation." This literature review aimed to identify articles published in English between 2012 and 2020 that addressed detoxification methods and other potential therapeutic uses of superoxide dismutases (SODs).

Kazlou, Aliaksandr. (2020). When nitroblue tetrazolium is available. Which is formed when adrenaline (epinephrine) undergoes an autooxidation process in a pH-neutral or slightly acidic environment, to its advantage. By measuring the generation of diformazan at an absorption wavelength of 560 nm, one may calculate an approximate rate of reduction in the production of superoxide anion radicals per unit of time. The addition of nitroblue tetrazolium to the reaction mixture enables this to happen. When superoxide anion radicals reduce nitroblue tetrazolium, the resulting compound is diformazan.

Stephenie, Sarah et al., (2020). One enzyme that the body uses is superoxide dismutase, or SOD. An innovative strategy for improving health in the face of pathological conditions is to include superoxide dismutase (SOD) from plants in the diet of mammals. There is a lack of studies on the potential of adding plant-derived superoxide dismutase (SOD) to animal diets as a health supplement because of the limited bioavailability of SOD when taken orally.

Mishra, Panchanand & Sharma, Pallavi. (2019). Reactive oxygen species (ROS) are byproducts of many plant metabolic processes that result in the early loss of electrons from various components of the electron transport chain, such as chloroplasts, mitochondria, and plasma membranes, to oxygen. Excess reactive oxygen species (ROS) are produced by abiotic conditions such as dryness, salt, metal toxicity, and high-temperature freezing. These variables disturb homeostasis and induce oxidative stress in plants. Two dangerous superoxide anion radicals ($O2^-$) are disproportionately produced by superoxide dismutases (SODs), metalloenzymes. Hydrogen peroxide and molecular oxygen are the less harmful byproducts of this process. So, SODs are considered the first line of defense against oxidative stress brought on by various abiotic factors. The presence or absence of metal cofactors at the active site allows plants' superoxide dismutases (SODs) to be classified into three groups: Cu-ZnSOD, MnSOD, and FeSOD. Each of these groups is responsible for a different task depending on the location of the SOD in the cell.

Li, Jine et al., (2019). The goal of this meta-analysis and literature review was to examine the monitoring and evaluation of superoxide dismutase (SOD) activity and its therapeutic value in the prevention and treatment of gastric cancer. Topic and Method To compile the data for the review, we searched the following databases: PubMed, Embase, Ovid, and the China National Knowledge Infrastructure (CNKI). Research that compared individuals with increased superoxide dismutase (SOD) activity in peripheral blood samples to healthy controls is part of this search. In order to refine our search, we used the terms "superoxide dismutase" and "gastric cancer." This research followed all of the guidelines laid forth by PRISMA, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Out of the ten randomized controlled trials that were located, six looked at levels of serum superoxide dismutase (SOD), three at levels in red blood cells, and one at levels in whole blood. A meta-analysis using the standardized mean difference (SMD) and 95% confidence interval (CI) found that gastric cancer patients had substantially lower superoxide dismutase (SOD) activity than healthy controls (SMD, -0.840; 95% CI, -1.463 to -0.218; p=0.008).

Dreyer, Bernd & Schippers, Jos. (2019). An important antioxidant enzyme present in every known kingdom of life is superoxide dismutase, or SOD. Its birthdate suggests it had a significant role in the evolution of life on Earth. The metal-cofactor that each enzyme in the superoxide dismutase (SOD) family uses allows for further classification into many classes. The function and structure of the most recent enzyme, copper-zinc superoxide dismutase (CuZnSOD), are the primary topics of this article. The development of CuZnSODs and CCS proteins are both covered in this article.

Younus, Hina. (2018). The antioxidant defense system of the body, which includes superoxide dismutases (SODs), is vital in protecting cells from free radical damage. When used medicinally, the enzyme is an effective weapon against illnesses caused by ROS. This article explores the many physiological and pathological situations that superoxide dismutase (SOD) may help with therapeutically. Ischemia, cancer, rheumatoid arthritis, neurological diseases, diabetes, cystic fibrosis, and many others fall within this category.

Saibi, Walid & Brini, Faïçal. (2018). It is possible to create oxidative stress by a variety of abiotic stressors. Reactive oxygen species (ROS) such hydrogen peroxide (H2O2) and superoxide (O2•-) may be produced in response to salt stressors and dehydration. This pair of species is formed by oxidoreductases such lipoxygenases, peroxidases, NADPH oxidase, and xanthine oxidase, which are involved in a wide variety of cellular processes. Plants have developed a wide array of antioxidant mechanisms, including enzymatic and non-enzymatic antioxidants, to control the production of reactive oxygen species (ROS) in reaction to stress.

Vuori, Kristiina & Kanerva, Mirella. (2018). The superoxide dismutase (SOD) activity was assessed using homogenates of several organisms, including cladocerans (Eubosmina maritima), pteropods (Limacina helicina), copepods (Limnocalanus macrurus, Acartia sp., Eurytemora affinis, Temora longicornis, and Calanus sp.), and algae (Limnocalanus sp.). The effectiveness of superoxide in preventing the generation of formazan dye from the reduction of a water-soluble tetrazolium salt (WST-1) is used as an indirect measure of superoxide dismutase (SOD) activity in this experiment. We know that this experiment took place because of Ukeda et al. (1999).

Berwal, Mukesh & Ram, Chet. (2018). A key function of the ubiquitous metalloproteases known as superoxide dismutases (SODs) is to neutralize reactive oxygen species (ROS). As an enzyme, it is vital in biological systems for neutralizing superoxide radicals. It does this by easing the dismutation process, which breaks down superoxide radicals into the oxygen peroxide and hydrogen peroxide they produce. Numerous SOD isoforms, such as Cu/Zn-SOD, Mn-SOD, and Fe-SOD, are present in most plant species. These SOD isoforms' active sites include distinct metal ions. Superoxide dismutase (SOD) activity and the number of its isoforms are positively correlated with plant tolerance levels, according to many research.

Kaushik, Rakesh et al., (2018). There are many ways in which superoxide radicals harm cells. These include lipid peroxidation in cell membranes, DNA damage, and the inhibition of essential enzyme activity. Superoxide dismutases (SOD) are enzymes that are essential for controlling heat stress and oxidative stress. During the peak heat stress period, this study set out to investigate the superoxide dismutase (SOD) activity in Jamunapari goats, both in the mother and her growing young. Reason being, SOD controls cellular defense mechanisms in response to heat stress. We were able to determine which genotypes in adult goats were susceptible to or resistant to heat stress by looking at the range of respiratory rate (RR) and heart rate (HR). Finding animals with RR values of 50 or higher and HR values of 130 or higher is one approach to identify those with a heat stress sensitive (HSS) phenotype. On the other hand, those with RR $\leq$30 and

HR ≤100 were shown to have a heat stress tolerant (HST) phenotype. In contrast to 9-month-old infants, 31.63% of nursing goats had their superoxide dismutase (SOD) activity inhibited.

Gopal Mrsb, Rajesh & Elumalai, Sanniyasi. (2017). Companies may now produce chemicals with better levels of safety, affordability, intensity, and specificity thanks to recent advances in biotechnology. The arsenal of contemporary chemicals is being supplemented with catalysts for cancer prevention agents, which are now under development. In terms of the quantity of research and innovation in which they are involved, they are now surpassing all other molecules. Superoxide dismutase (SOD), a cell-reinforcing chemical, was commercially available in the 1990s.

Ighodaro, Osasenaga & Akinloye, Oluseyi. (2017). Endogenous enzymatic and non-enzymatic antioxidants form a comprehensive antioxidant defense grid that the body may use to protect itself against free radicals. When these molecules come together, they create a shield that stops free radicals from destroying vital biomolecules and, eventually, the tissues of the body. Antioxidants may be classified as first-line, second-line, third-line, or even fourth-line protection antioxidants depending on their overall reactions to free radical invasion. Antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) serve as the body's initial line of defense against the superoxide anion radical (*O2), which is continually generated in normal metabolism, particularly through the mitochondrial energy production pathway (MEPP).

Unlu, Ezgi & Takaç, Serpil. (2017). Raising the activity of the superoxide dismutase (SOD) enzyme—an important component of the antioxidant defense system—provided ideal circumstances for the development of Rhodotorula glutinis. According to Central Composite Design, the optimal parameters for achieving a superoxide dismutase (SOD) activity of 2.40 U were a pH of 5.47, a temperature of 33.9 ⁽C, and 16.50 g L·1 glycerol. A carbon source that is also a byproduct of biodiesel manufacturing, glycerol, was used for this in order to do it. Superoxide dismutase (SOD) activity was already high, so scientists investigated several radical promoters to provide it an extra boost. A protein level of 21.57 U mg¡1 and a 1.6-fold increase in SOD activity were observed in methyl viologen, which showed the greatest enzyme activity. The effect of dissolved oxygen (DO) on superoxide dismutase (SOD) activity was also assessed using a variety of bioreactor operating techniques. The sole way to boost superoxide dismutase (SOD) activity, which led to 75 U mg¡1 protein activity, was to provide a high concentration of dissolved oxygen (DO) starting at the commencement of the culture phase.

Mukherjee, Monalisa et al., (2017). Superoxide dismutase (SOD) has been the subject of many recent scientific research that have detailed its potential therapeutic uses in the fight against oxidative stress and cancer. Clinical and preclinical studies have been used to carry out these research. Indeed, several kinases, molecular signaling pathways. The therapeutic use of superoxide dismutase (SOD) as a possible medication is still not completely understood, despite the large quantity of existing research, patents, clinical trials, and marketed products. We brought novel formulation methodologies including gene manipulation, nano-formulations, and click chemistry; we investigated the structural organization and cellular signaling; we discovered a substantial relationship with kinase; and we explored new target locations.

Deus, K.E. et al., (2015). Reduced yields from upland rice agriculture are caused in large part by drought in many parts of the world. In addition, after boosting the formation of reactive oxygen species (ROS), it activates a complex antioxidant process. Researchers in this study examined the levels of eight different isoforms of superoxide dismutase (SOD) and their enzymatic activity in upland rice cultivars that were either drought-tolerant or drought-sensitive. This pertains to the Primavera and Douradão varieties of Oryza sativa japonica. In addition, the study examined the plant's shoot and root tissues throughout two growth periods, one in which the plant was watered regularly and the other in which it was watered less often. Spectrophotometry was used for the evaluation of SOD activity, while quantitative polymerase chain reaction (qPCR) tests were utilized for the investigation of gene expression. Superoxide dismutase (SOD) enzymatic activity (268.00 SOD UN mg-1) significantly increased in the tolerant cultivar's root tissue throughout the reproductive stage ($p < 0.05$). Additionally, the sensitive cultivar exhibited an increase in SOD enzymatic activity in its root (172.56 SOD UN mg-1) and leaf (112.17 SOD UN mg-1) tissues throughout the reproductive stage.

Materials and Methods

Procurement and maintenance:

The fish species included in this research taken from Bindeshwari Fishery pond, Bhilampur, Jaunpur district. The fish were transported to a lab and then placed in glass aquariums for release. The fishes were given bug larvae to eat and given a week to adjust to the aquarium environment. Two times daily, hypoxic conditions were prevented by aerating the water. Fish of a consistent size (around 100 gm) were subjected to sublethal dosages of Atrazine for 96 hours in order to carry out the histopathological examinations. Over the course of 96 hours, the deadly amounts of Atrazine, Contaf, and Fenvalerate were 6.521ppm, 3.9711ppm, and 3.6271ppm, respectively. A control group and an experimental group were each given a different set of fish. Atrazine, Contaf, and Fenvalerate were administered to 10 fishes at sublethal doses, respectively. The fish that had already died were then promptly removed. As a control, ten fish were maintained in water without any kind of treatment. Following After the sublethal and control groups' exposure periods came to a close, the fish were weighed and then dissected to remove the kidney, gonads, and liver. Weighing the liver and gonads was the first step in further processing the samples for histology.

To fixate the cell structures while preserving their morphological identity, organs such as the kidney, liver, and gonads were immersed in Bouin's fixative for twenty-four hours after dissection. To stop autolysis, fixatives make tissue proteins intractable and change the cell's natural jelly-like state—whether it's alive or fixed—into a fine granular spongy mass. Denaturation of proteins alters the tissue's texture and reactivity, which in turn causes a change during fixation. In a 75 ml container, combine the picric acid, formaldehyde, and acetic acid until the acid is completely dissolved. makes up the aqueous Bouins fluid, which is used for tissue fixation. Since picric acid may interfere with the staining procedure, it was rinsed out of the gonads, liver, and kidney after fixation using room temperature running tap water.
Dehydration was used to eliminate water from the fixed tissues once the fixing phase was finished. Using progressively higher grades of alcohol—30%, 50%, 70%, 90%, and 100%—to gradually remove and replace water from tissues is known as dehydration. This method is highly significant for dehydrating tissues because it prevents putrefaction. Cell membranes are shielded from harm and cellular abnormalities are prevented. After two minutes of submersion in absolute alcohol, tissues were removed and replaced with new alcohol for a total of 10 minutes. Room temperature was also used for dehydration.

Clearing of Tissues - When a person becomes dehydrated, their tissues get saturated with alcohol. To prepare the tissues for sectioning, they were dehydrated and then treated with paraffin wax. In order for the paraffin to fill in for the alcohol, the tissue must first be removed. To compensate for the alcohol, paraffin cannot be diffused into the tissue as the two substances are insoluble in one other. Hence, the tissue must undergo an intermediate phase following dehydration: being immersed in a solution that is able to dissolve in alcohol while yet preserving the paraffin to penetrate the tissue. "Clearing" describes this stepping stone. It is usual practice to employ the cleaning chemical xylene to cleanse the tissue. In addition to causing paraffin to penetrate tissues, this process removes their opacity, making them visible. For five minutes, the tissues were immersed in xylene. Ten minutes apart, two xylene changes were administered. Afterwards, they spent two hours at ambient temperature before being undergo cold infiltration in a wax and xylene combination. Xylene not only makes tissue translucent, but it also causes paraffin to penetrate the tissue.
Embedding and Block Making—Standardizing tissue has to be embedded in order to be examined under a microscope using a microtome for sectioning. The unique properties of paraffin wax make it an ideal material for the embedding process. A combination of aliphatic hydrocarbons makes it up. Soaking the tissues in molten wax at a consistent temperature that coincided with the embedding medium's melting point was done during the hot infiltration process. Two changes of molten paraffin wax from 52o C to 54o C were used for a ten-minute hot infiltration process. For improved impregnation, all tissues were subjected to three or four wax changes. Following the embedding of the tissue in wax, it was cast into a paraffin block. When manufacturing blocks, great care is required to ensure that the wax is properly reinforced on all sides of the tissues. We prepped, cut, and chilled the blocks for the night. The sections were cut using a rotary microtome at a thickness of 7 microns.

Staining
Haematoxylin and Eosin (HE):
1) Using alcohol grades, sections were deparaffinized in xylene and then brought down to water.
2) Delafield's haematoxylin stained them.
3) After that, they were rinsed for fifteen minutes under running water from the faucet.
4) They were subsequently rendered 90% dry by application of graded alcohol.
5) 49 percent eosin (Gurr) in 90 percent alcohol was used as a counterstain.
6) The slides were washed twice with 90% alcohol.
7) Subjected to two cycles of 100% alcohol to dehydrate.
8) They were rinsed in two 30-minute xylene changes before being mounted in DPX.

Result and Discussion
Here are the histological abnormalities reported in *Labeo rohita* liver, kidney, testis, and ovary after being exposed to Fenvalerate, Atrazine, and Contaf: -

Liver: Control
Liver histology from the control fish, *Labeo rohita*, revealed polygonal hepatic cells (hepatocytes) with a rounded nucleus in the middle and a visible nucleolus, as well as fine, transparent cytoplasm. Hepatic tubules do not, however, partition the liver. Organized into cords are the hepatic cells. Since the liver is a haemapoetic organ, there were many sinusoids where the blood cells could be clearly seen. The presence of cuboidal cells lining the inner surface of the few bile ducts that were seen was accompanied by a fibrous connective tissue layer that included many cell types, including Kupffer cells, fat-storing cells, macrophages, and pericytes. Pancreatic tissue implanted in the liver is also seen in the slice.

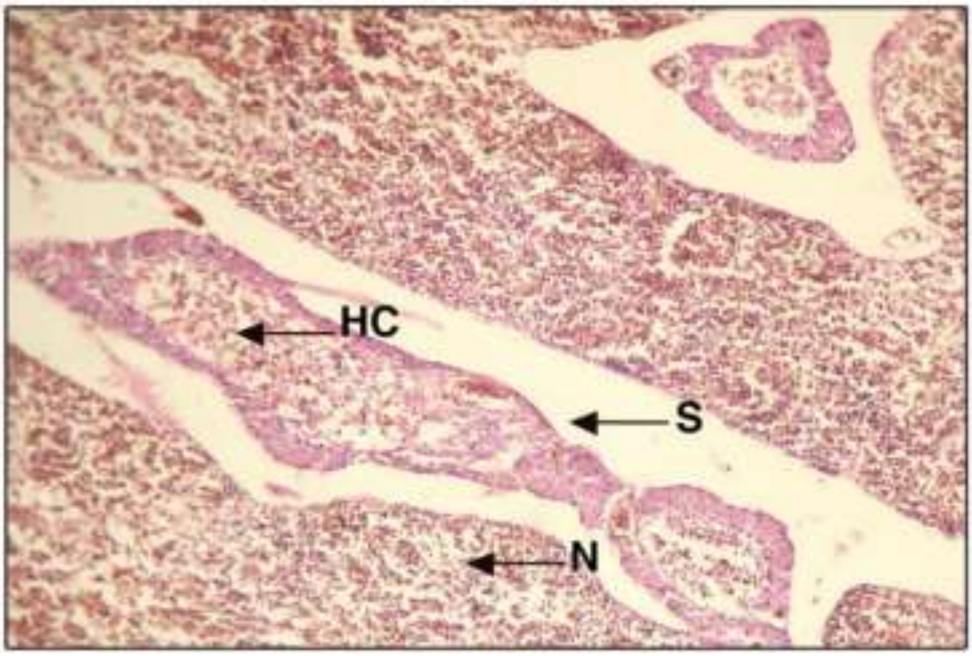

Figure 3.1 Transverse Section of liver of *Labeo rohita* exposed from control fish 200x

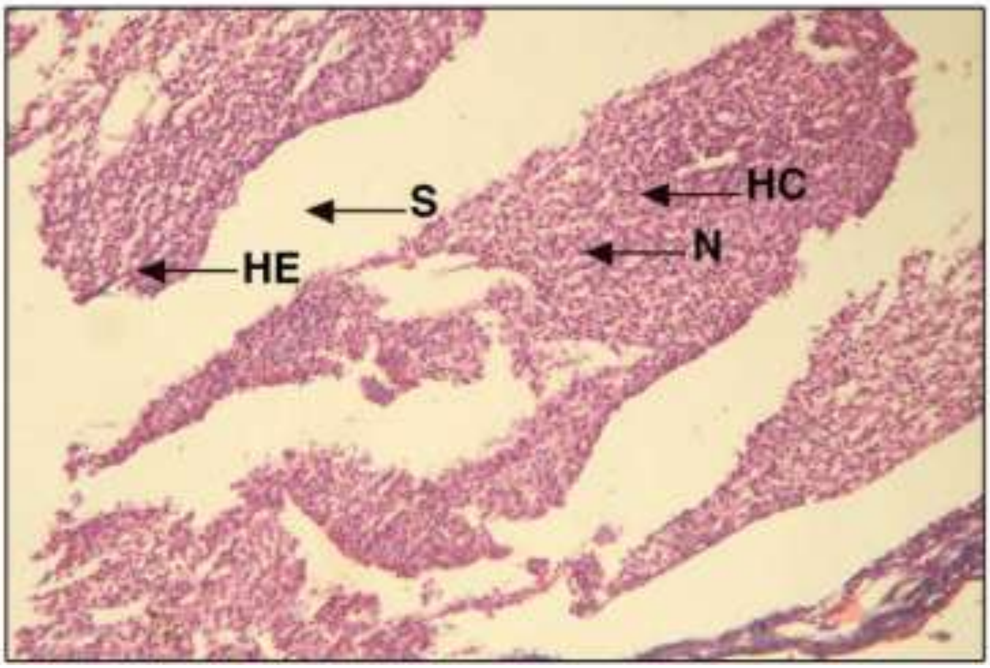

Figure 3.2 Transverse Section of liver of *Labeo rohita* exposed to fenvelarate for 24hrs 200x

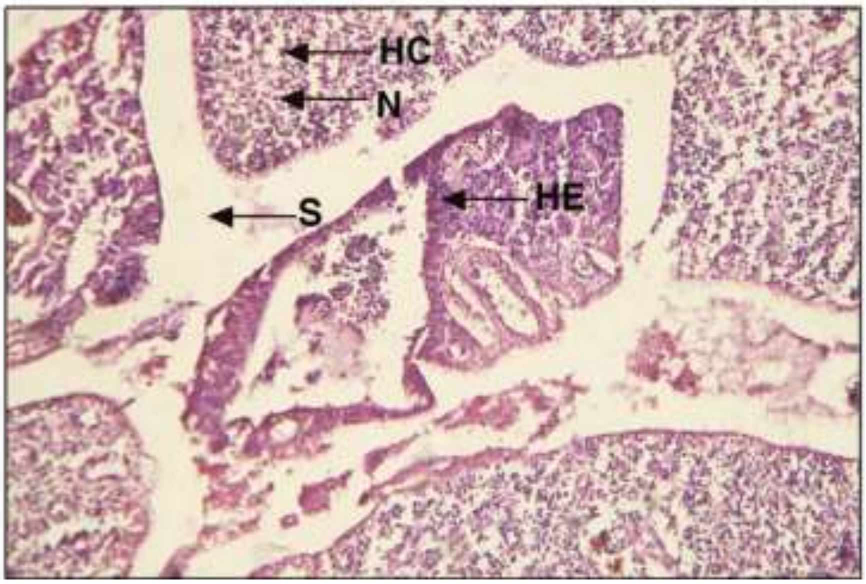

Figure 3.3 Transverse Section of liver of *Labeo rohita* exposed to fenvelarate for 48hrs 200x

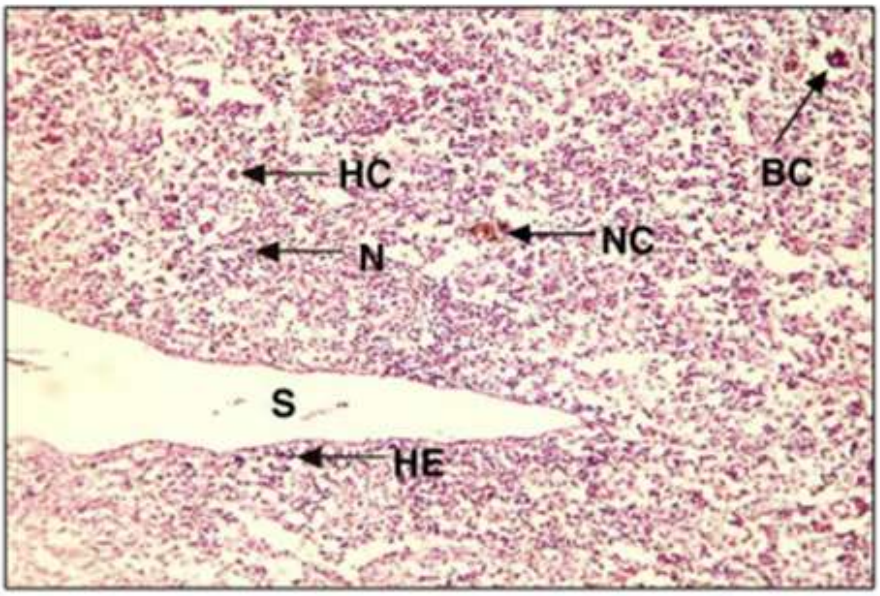

Figure 3.4 Transverse Section of liver of *Labeo rohita* exposed to fenvelarate for 72hrs 200x

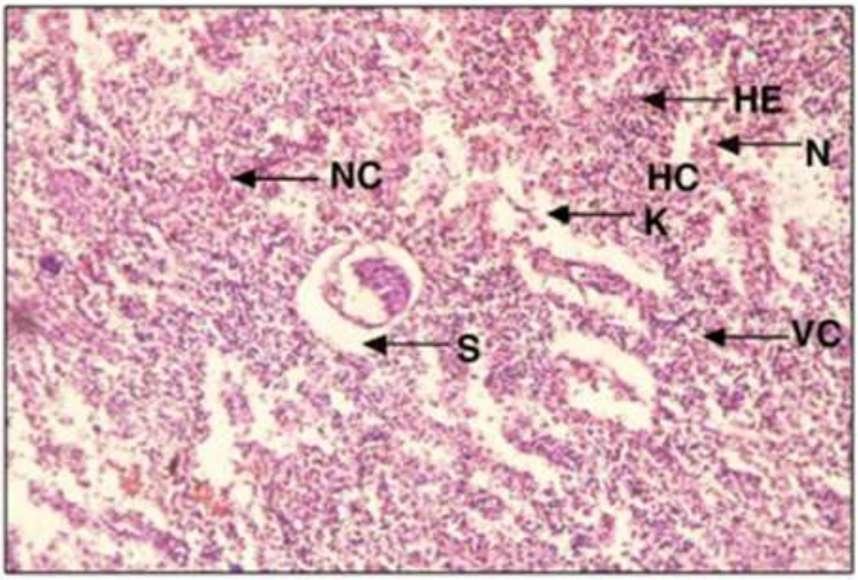

Figure 3.5 Transverse Section of liver of *Labeo rohita* exposed to fenvelarate for 96hrs 200x

Experimental liver

After 96 hours of treatment with a sublethal dosage of Fenvalerate, the liver exhibited signs of necrosis, hepatocyte degradation, ductal cell proliferation, and the formation of tiny vacuoles. This finding is consistent with what Saxena et al. (1989) found. Malathion is more harmful to the liver of Channa punctatus than carboryl and denova lipid production, according to their findings.

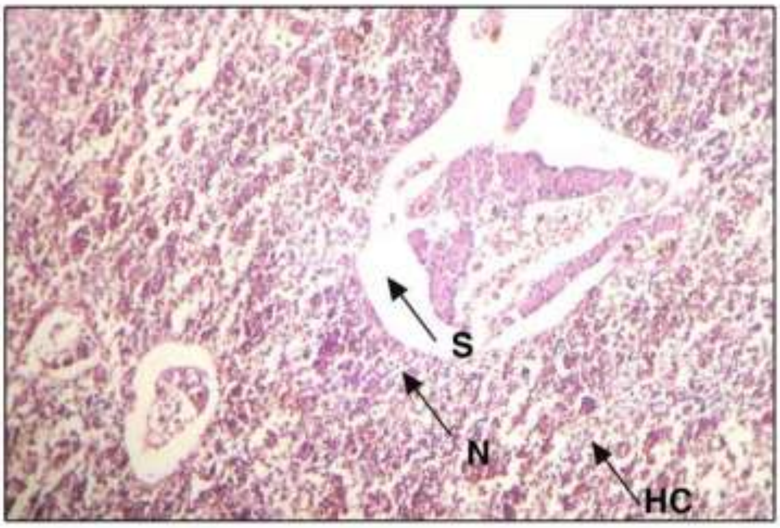

Figure 3.6 Transverse Section of liver of *Labeo rohita* control 200x

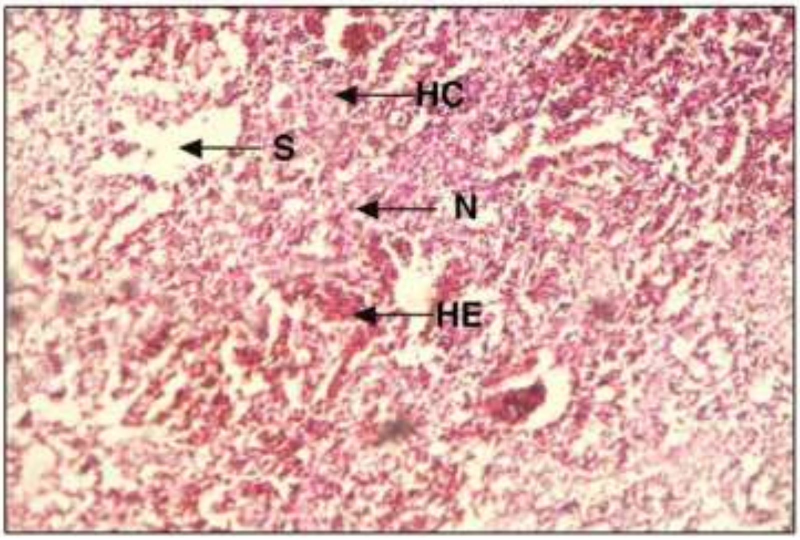

Figure 3.7 Transverse Section of liver of *Labeo rohita* exposed to atrazine for 24hrs 200x

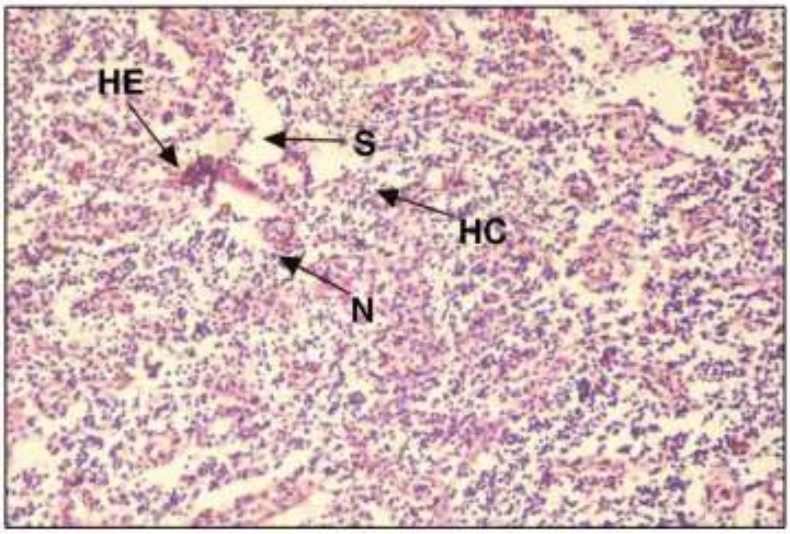

Figure 3.8 Transverse Section of liver of *Labeo rohita* exposed to atrazine for 48hrs 200x

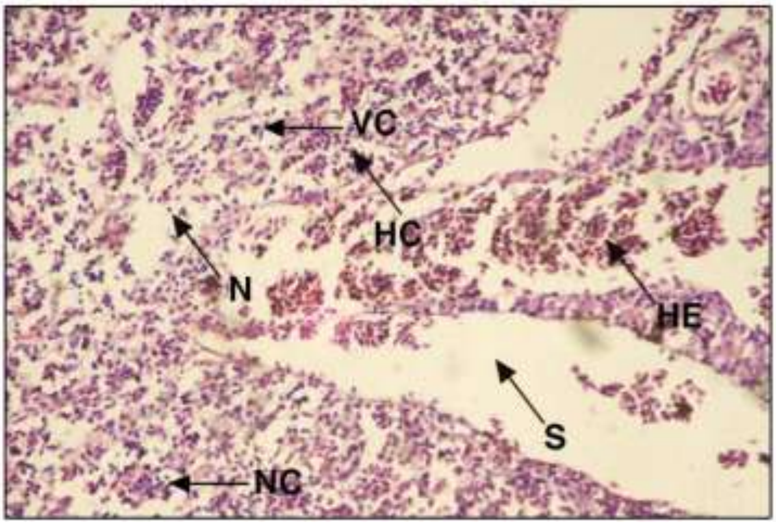

Figure 3.9 Transverse Section of liver of *Labeo rohita* exposed to atrazine for 72hrs 200x

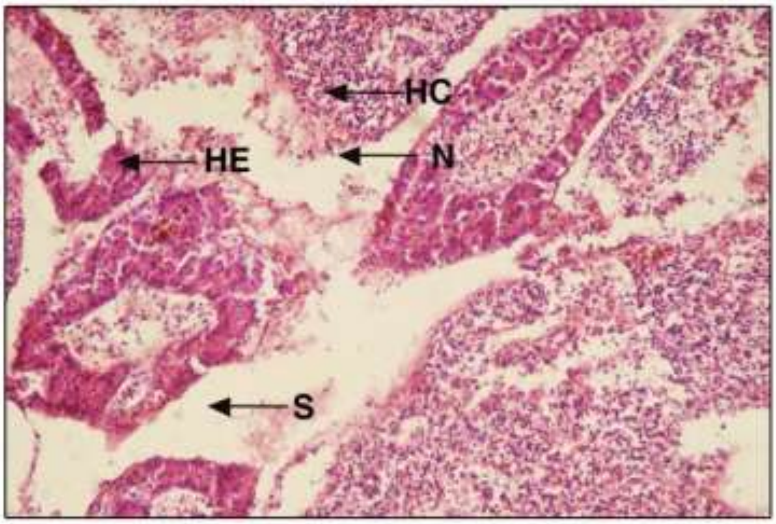

Figure 3.10 Transverse Section of liver of *Labeo rohita* exposed to atrazine for 96hrs 200x

There is necrosis, hepatic cell scattering, proliferation of ducted cells, as well as the development of small vacuoles inside the liver after 96 hours of administration with a sublethal dosage of Atrazine. Degeneration of hepatic cells due to nuclei displacement is also revealed. The hepatic cords are in a state of disarray. Likewise, tiny vacuoles appeared in the liver after 96 hours of treatment with a sublethal dosage of Contaf, along with hepatocyte degradation, cell necrosis, ductal cell growth, and necrosis.

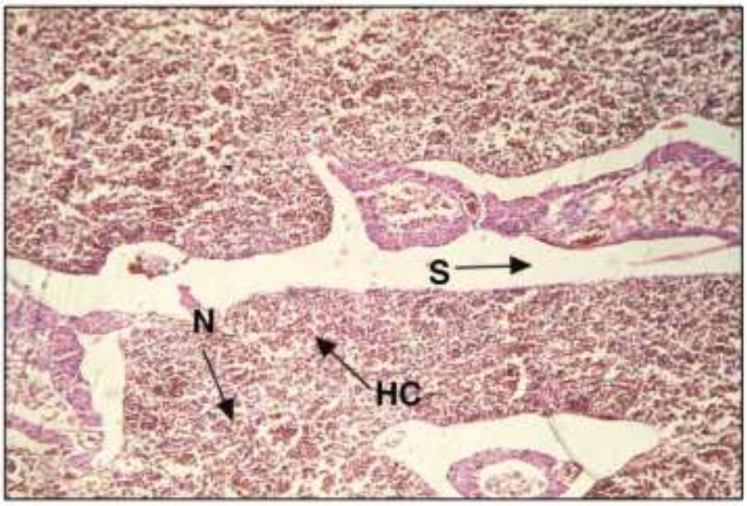

Figure 3.11 Transverse Section of liver of *Labeo rohita* control 200x

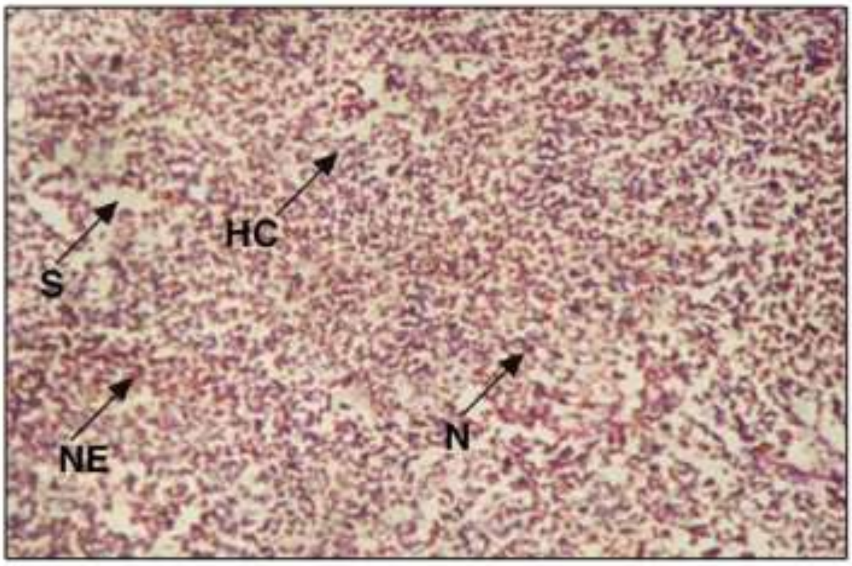

Figure 3.12 Transverse Section of liver of *Labeo rohita* exposed to contaf for 24hrs 200x

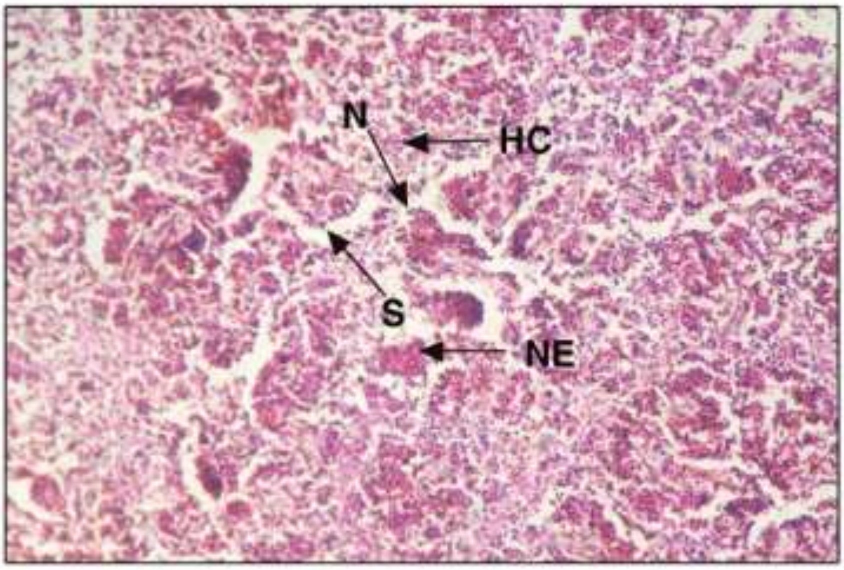

Figure 3.13 Transverse Section of liver of *Labeo rohita* exposed to contaf for 48hrs 200x

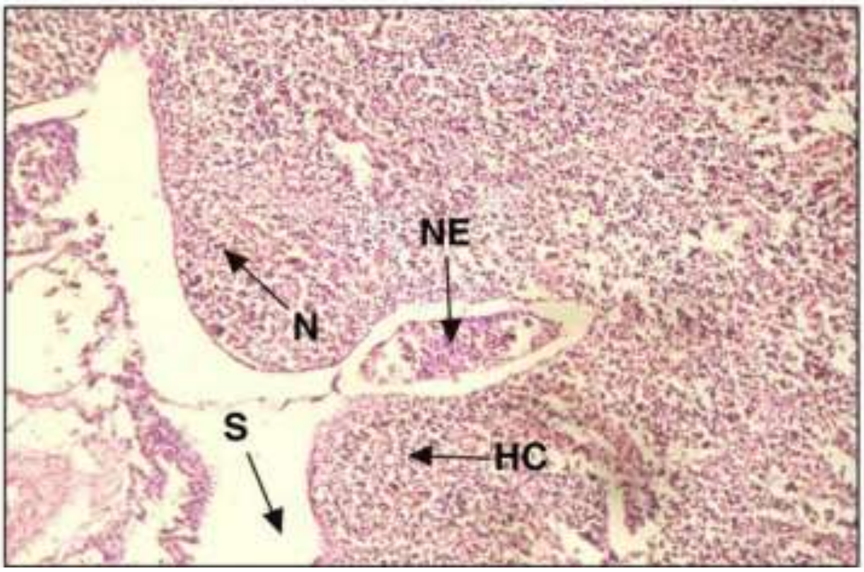

Figure 3.14 Transverse Section of liver of *Labeo rohita* exposed to contaf for 72hrs 200x

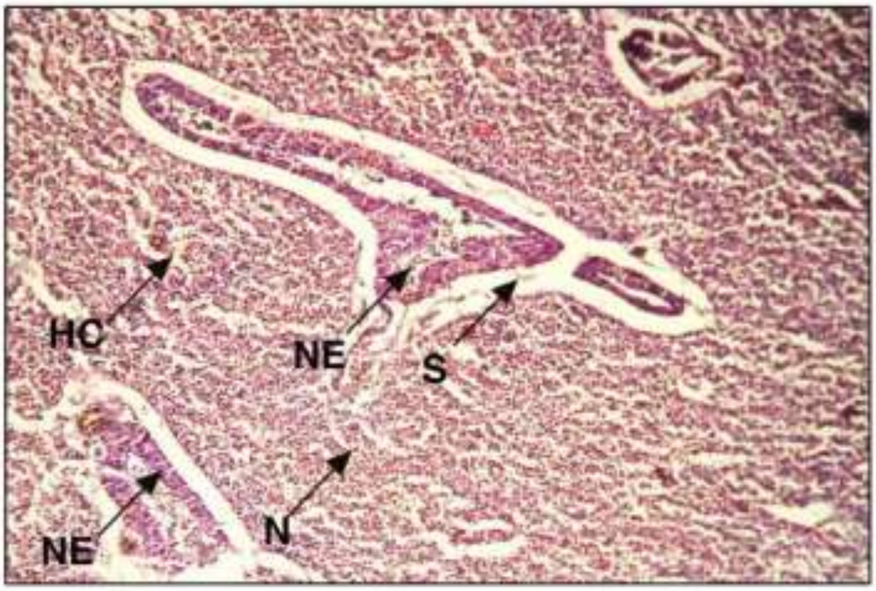

Figure 3.15 Transverse Section of liver of *Labeo rohita* exposed to contaf for 96hrs 200x

Control Kidney-

Pathology in general: - Both the head and the body make up the teleostean kidney. The lymphoid tissue that makes up the head kidney is located at the front of the kidney. The body's primary hematopoietic tissue is located in the interstitial space. The glomerulus and urine tubule are the two primary components of a nephron. An inner layer of single, flattened epithelia makes up the glomerular capsule. One layer of epithelial cells makes up renal tubules. The interstitial area between the glomerular capillary loops is filled with mesangium. The neck segment of the kidneys has very small and delicate tubules. Splitting the proximal convoluted section in half, we get segments I and II. Microvilli are tightly packed within the tubular lumen of cuboidal epithelial cells that make up the renal tubules. Segment II of the kidneys contains cuboidal epithelial cells that line the tubules. Inside the tubules, you may see cilia and microvilli. Epithelial cells lack microvilli in the distal convoluted section. The amount of coiled uriniferous tubules may be seen in the fish's kidney that served as a control. The proximal segment is characterized by columnar epithelial cells that contain a nucleus. The nuclei stand out and have a spherical shape. An alkaline membrane encircles the distal tubules. Nested between the tubules are tissues that produce blood. There were several nephrons visible in the Transverse Sections, as well as the glomeruli. A nephron has two components, the urine tubule and the proximal tubule; a healthy nephron has a separate glomerulus and a connecting tubule.

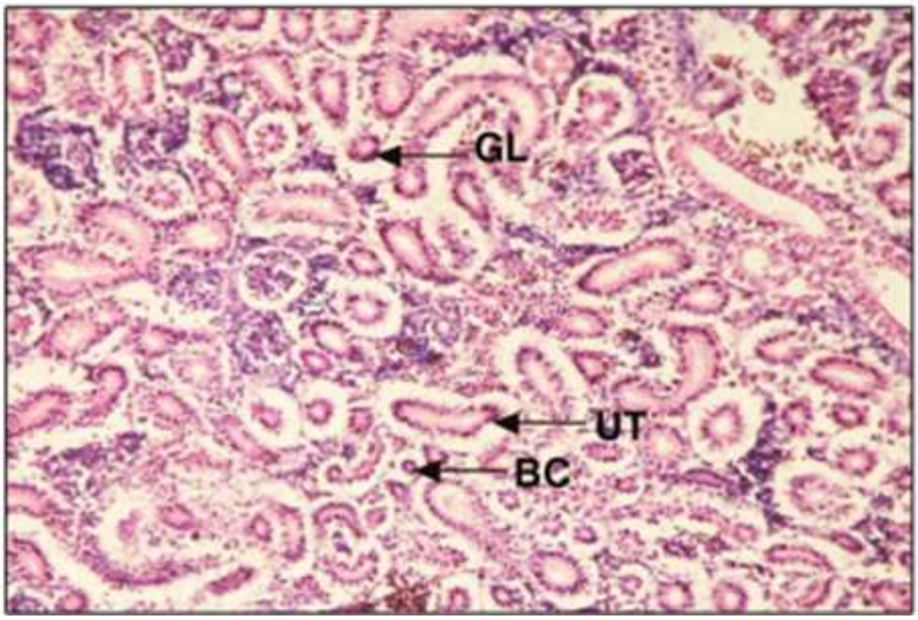

Figure 3.16 Transverse Section of kidney of *Labeo rohita* from control fish 200x

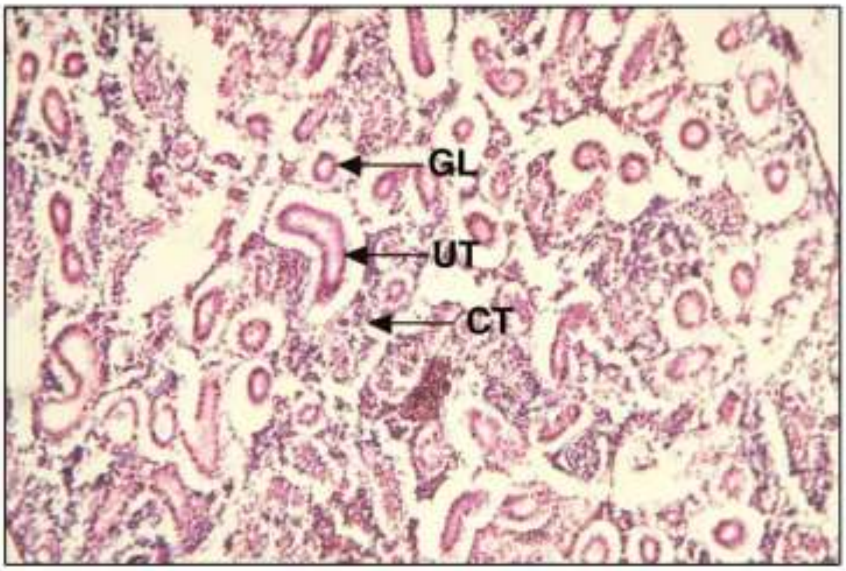

Figure 3.17 Transverse Section of kidney of *Labeo rohita* exposed to fenvelarate for 24hrs 200x

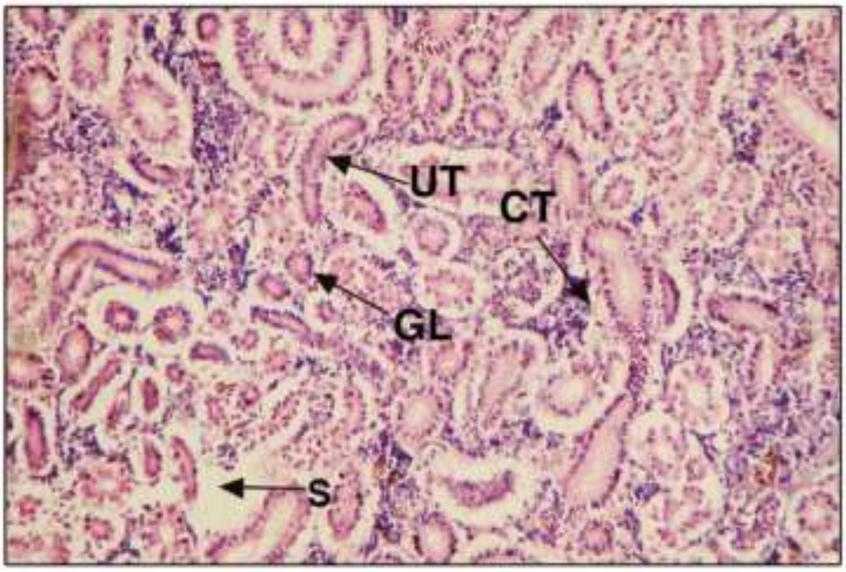

Figure 3.18 Transverse Section of kidney of *Labeo rohita* control 200x

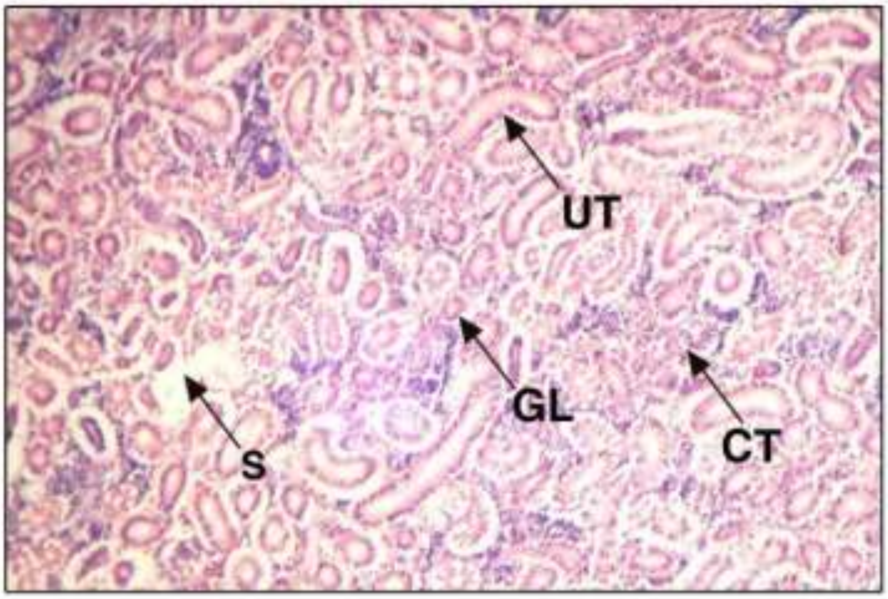

Figure 3.19 Transverse Section of kidney of *Labeo rohita* exposed to atrazine for 24hrs 200x

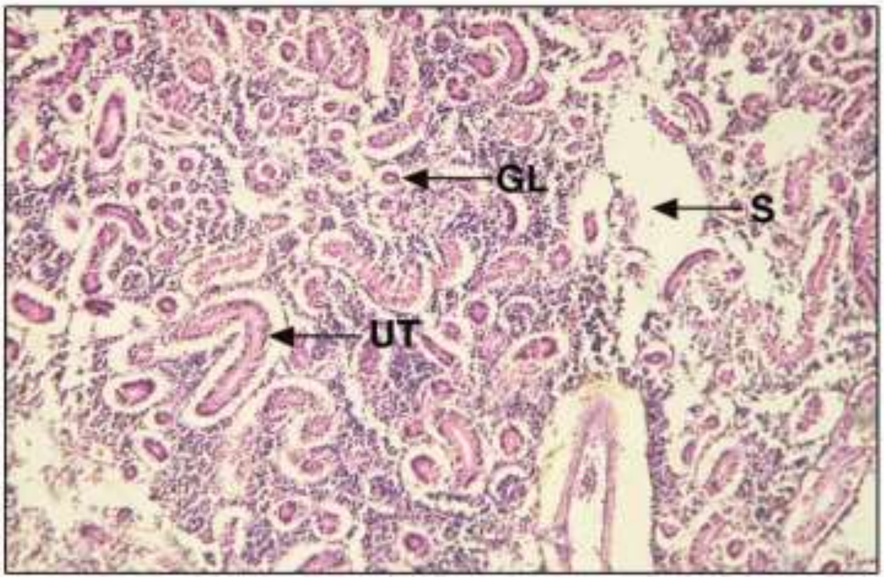

Figure 3.20 Transverse Section of kidney of *Labeo rohita* exposed to atrazine for 48hrs 200x

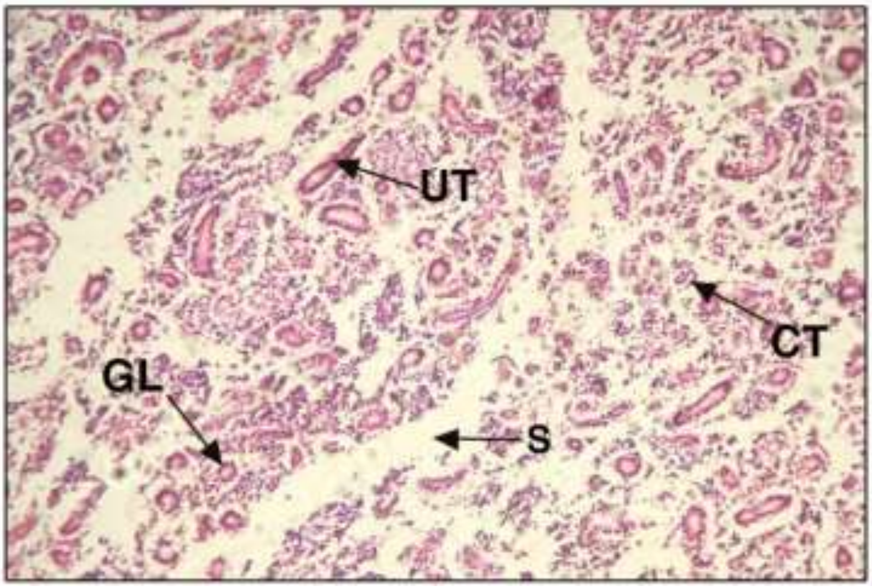

Figure 3.21 Transverse Section of kidney of *Labeo rohita* exposed to atrazine for 72hrs 200x

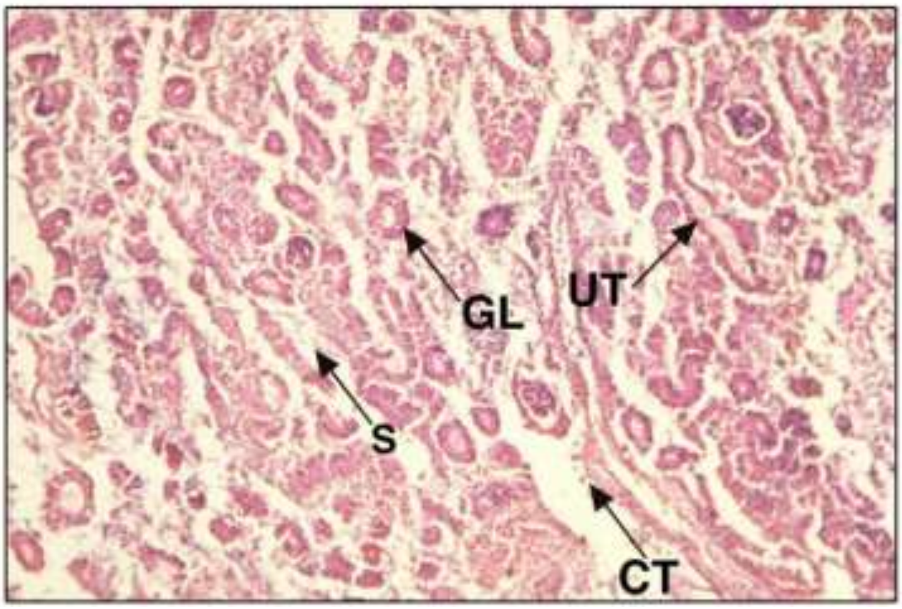

Figure 3.22 Transverse Section of kidney of *Labeo rohita* exposed to atrazine for 96hrs 200x

Experimental Kidney:

Degenerative alterations in the collecting tubule and hemopoetic epithelial cells and severe necrosis in the proximal tubules that forms vacuoles are caused by sublethal doses of Fenvalerate. Atrazine causes disrupted proximal and distal tubules and connective tissue sinuses at sublethal dosages. Symptoms seen after being exposed to a dosage below the deadly one of Contaf included swelling in the renal tubules, damage to the proximal and distal tubules, the formation of sinuses in connective tissue, necrosis, and other infections. After24,48,72, and 96 hours of exposure to sublethal doses of Atrazine, Contaf, and Fenvalerate, respectively, the histology of the experimental kidney revealed damaged structure, depending on the amount and duration of exposure.

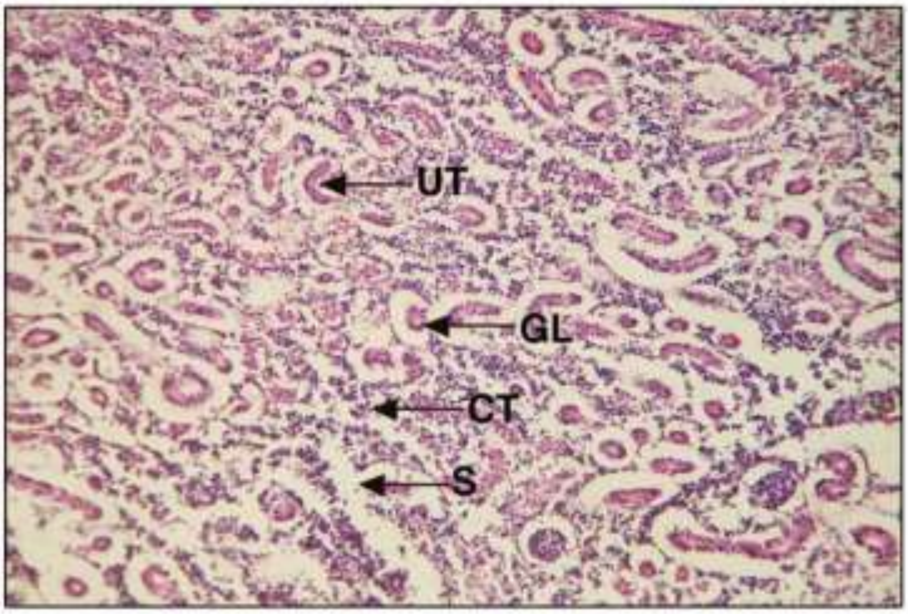

Figure 3.23 Transverse Section of kidney of *Labeo rohita* exposed to fenvelarate for 48hrs 200x

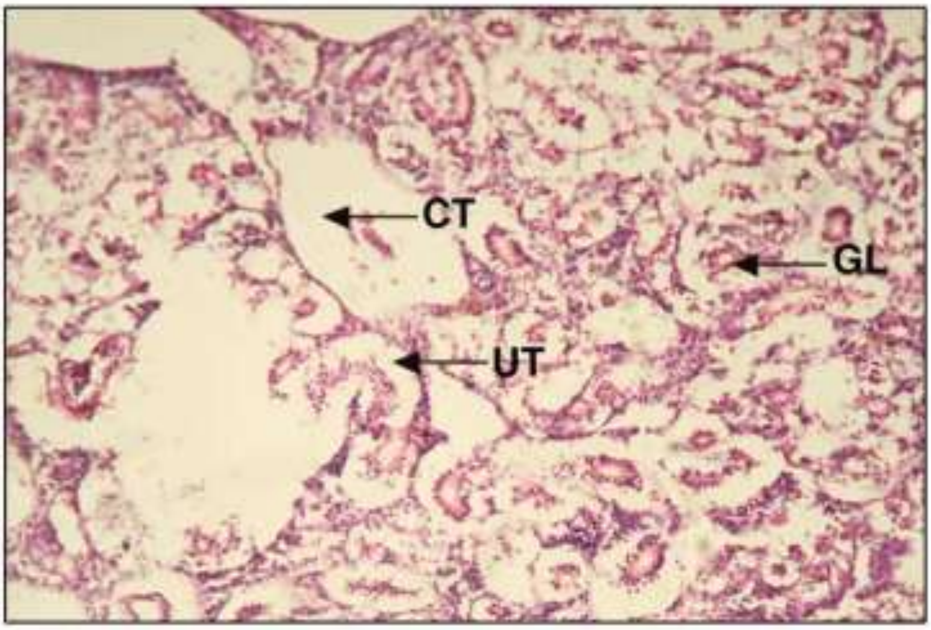

Figure 3.24 Transverse Section of kidney of *Labeo rohita* exposed to fenvelarate for 72hrs 200x

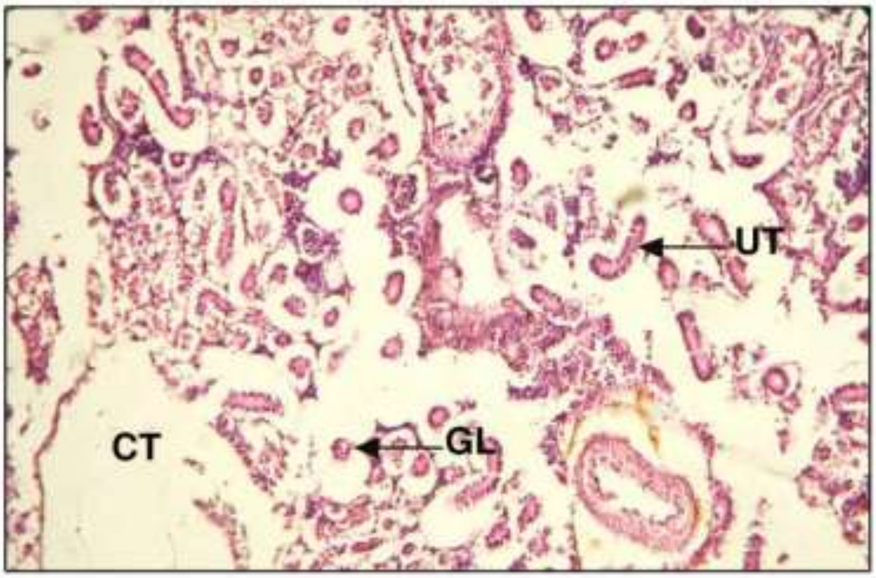

Figure 3.25 Transverse Section of kidney of *Labeo rohita* exposed to fenvelarate for 96hrs 200x

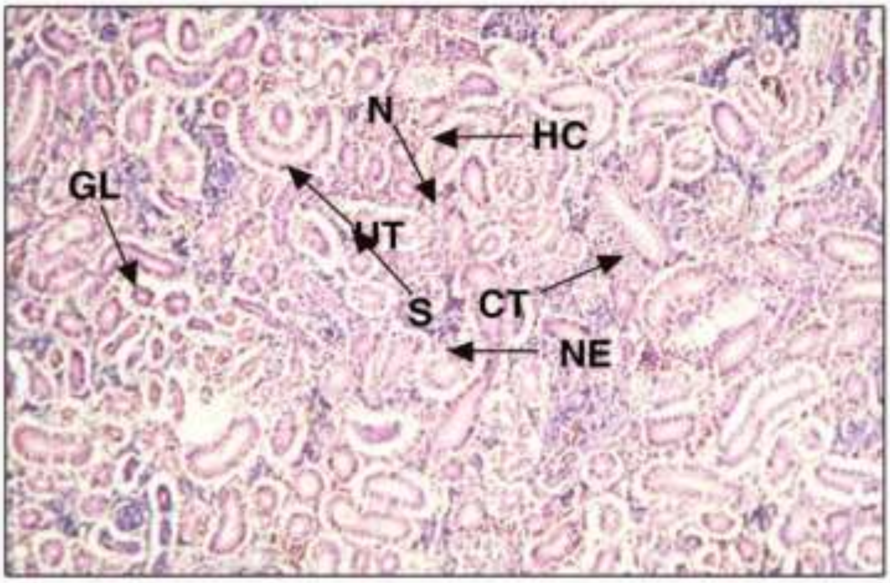

Figure 3.26 Transverse Section of kidney of *Labeo rohita* control 200x

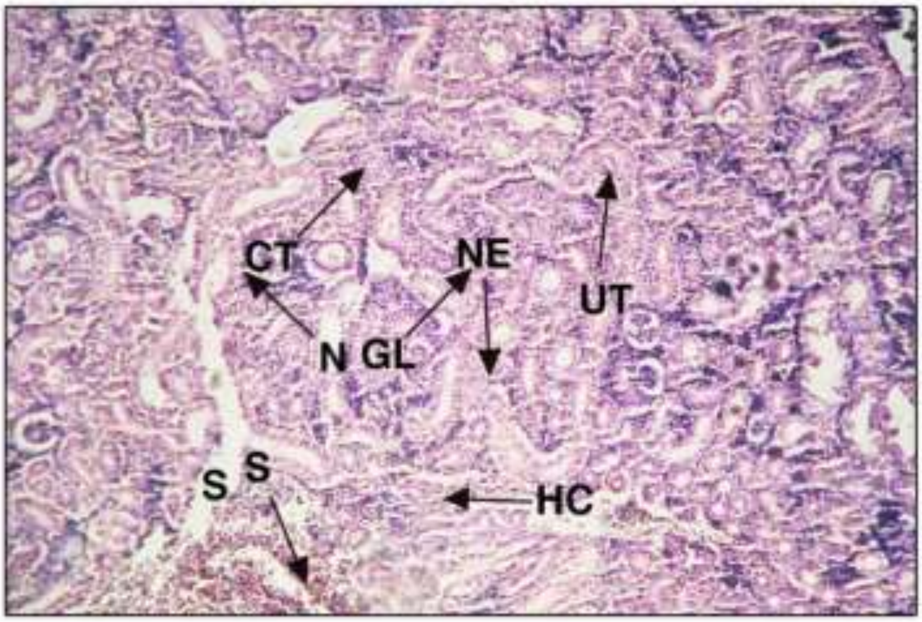

Figure 3.27 Transverse Section of kidney of *Labeo rohita* exposed to contaf for 24hrs 200x

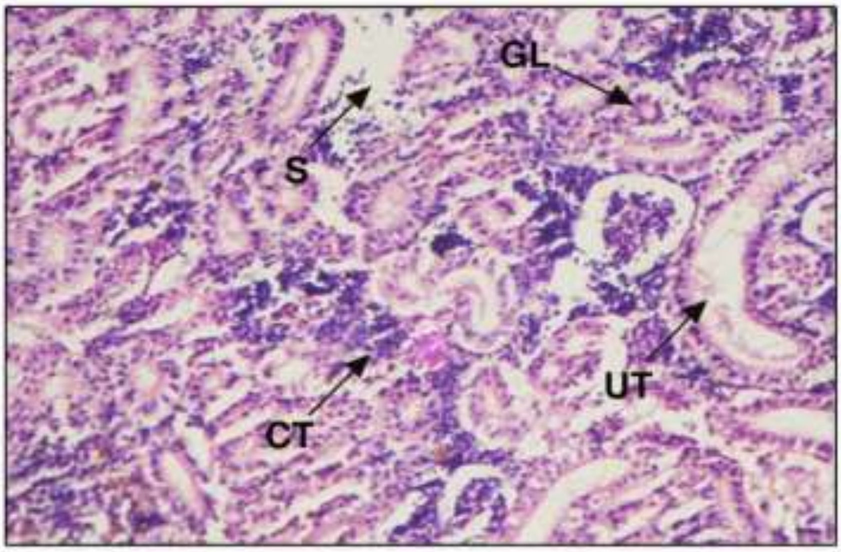

Figure 3.28 Transverse Section of kidney of *Labeo rohita* exposed to contaf for 48hrs 200x

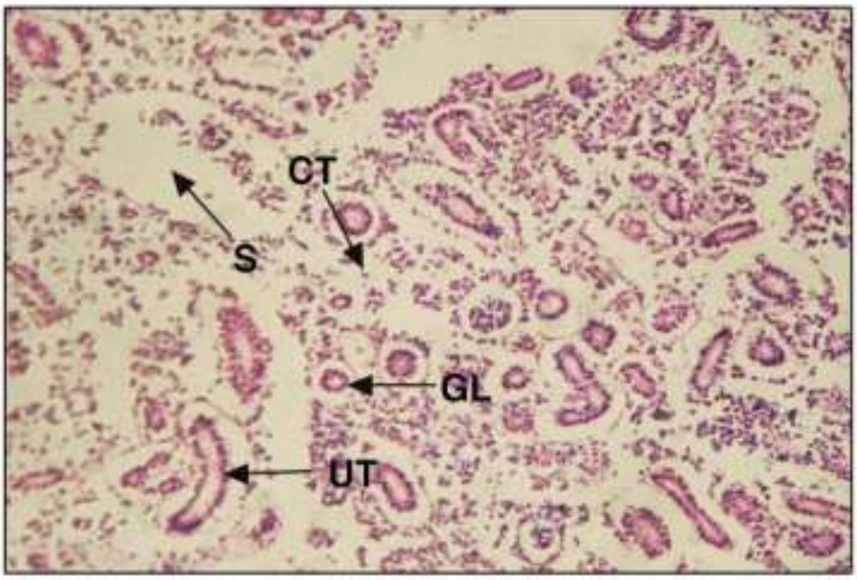

Figure 3.29 Transverse Section of kidney of *Labeo rohita* exposed to contaf for 72hrs 200x

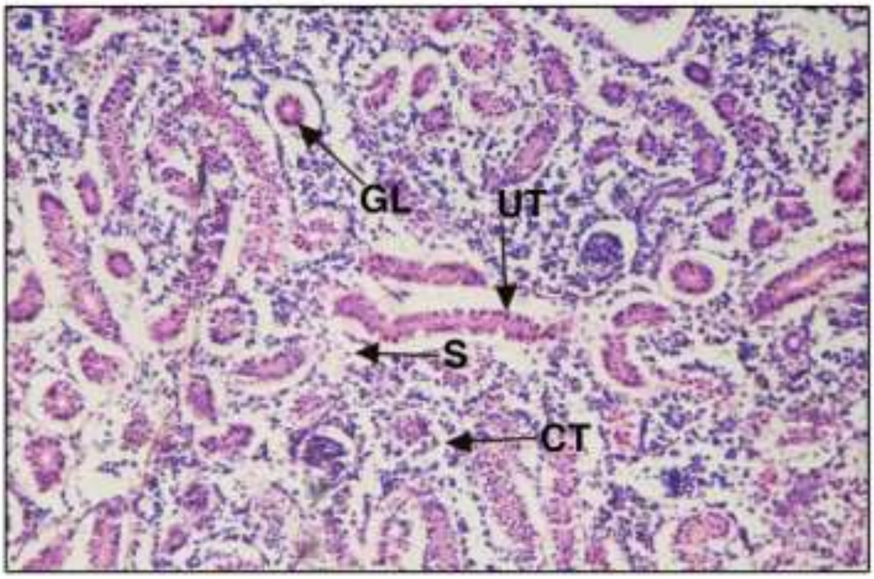

Figure 3.30 Transverse Section of kidney of *Labeo rohita* exposed to contaf for 96hrs 200x

Conclusion

Finally, studying the correlation between the histopathological hepatosomatic index (HSI) and superoxide dismutase (SOD) enzyme activity in freshwater teleost fish yielded important information on how physiological stress affects cellular structures and tasks. The liver might experience oxidative stress due to environmental factors such pollution or changes in water quality. This reaction was reflected in the HSI. Reactive oxygen species (ROS) are potentially damaging byproducts of cellular metabolism; when physiological stress levels are high, the body often responds by increasing superoxide dismutase (SOD) activity. Because oxidative damage may impact the structure and function of proteins within cells, superoxide dismutase (SOD) plays a crucial role in preserving cell integrity, which includes the liver. By investigating SOD activity alongside the HSI, researchers improved their knowledge of how oxidative stress impacts the health of teleost fish and the liver's adaptive responses to stress.

Utilizing a correlation between histological changes and SOD activity in teleost fish allowed for the further classification of new chemicals based on their potential environmental hazards. Substances that resulted in significant changes to the HSI and an increase in SOD activity were identified as potential environmental hazards as a way to assess the ecological impact of pollutants. The physiological responses of fish exposed to chemicals that harmed their livers and caused oxidative stress were monitored for the purpose of monitoring the state of the environment.

References

[1] A, Dr & M, Dr. (2022). Superoxide Dismutase (SOD): A Promising Enzyme in the Area of Biopharmaceuticals in Its Native and Immobilized Form: A Review. International Journal for Research in Applied Science and Engineering Technology. 1472-1478. 10.22214/ijraset.2022.46437.

[2] Batool, Raufa & Umer, Muhammad Jawad & Hussain, Babar & Anees, Muhammad & Wang, Zhenying. (2022). Molecular Mechanisms of Superoxide Dismutase (SODs)-Mediated Defense in Controlling Oxidative Stress in Plants. 10.1007/978-981-16-7981-0_8.

[3] Adhikari, Aniket & De, Madhusnata. (2022). Study of Superoxide Dismutase (SOD) Activity Among Betel Quid Chewers of Indian Population. Journal of Cancer Research and Therapeutics. 6. 1- 3. 10.31579/2640-1053/117.

[4] Karmakar, Arnab & Das, Abhishek & Ghosh, Noyel & Sil, Parames. (2022). Superoxide dismutase. 10.1016/B978-0-12-819096-8.00027-6.

[5] Rispandi, Marsal & Zulfahri, & Harahap, Novita. (2022). Effect of Regular Exercise and Antioxidant on Superoxide Dismutase (SOD). Kinestetik : Jurnal Ilmiah Pendidikan Jasmani. 6. 553-559. 10.33369/jk.v6i3.23751.

[6] Bharti, Sandhya & Rasool, Fazle. (2021). Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on Channa punctatus (Bloch) fish. Toxicology Reports. 8. 10.1016/j.toxrep.2021.02.018.

[7] Reddy, P.. (2021). ALTERATIONS IN HEPATOSOMATIC INDEX (HSI) AND CONDITION FACTOR (CF) IN *LABEO ROHITA* FROM ANTHROPOGENICALLY POLLUTED RIVER.

[8] Rosa, Arianna & Corsi, Daniele & Cavi, Niccolò & Bruni, Natascia & Dosio, Franco. (2021). Superoxide Dismutase Administration: A Review of Proposed Human Uses. Molecules. 26. 1844. 10.3390/molecules26071844.

[9] Kazlou, Aliaksandr. (2020). Determination of superoxide dismutase activity. 10.13140/RG.2.2.32125.18400.

[10] Stephenie, Sarah & Chang, Ying Ping & Gnanasekaran, Ashok & Me, Norhaizan & Gnanaraj, Charles. (2020). An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. Journal of Functional Foods. 68. 10.1016/j.jff.2020.103917.

[11] Mishra, Panchanand & Sharma, Pallavi. (2019). Superoxide Dismutases (SODs) and Their Role in Regulating Abiotic Stress induced Oxidative Stress in Plants. 10.1002/9781119468677.ch3.

[12] Li, Jine & Lei, Jun & He, Liyun & Fan, Xiude & Yi, Fengming & Zhang, Wenxiong. (2019). Evaluation and Monitoring of Superoxide Dismutase (SOD) Activity and its Clinical Significance in Gastric Cancer: A Systematic Review and Meta-Analysis. Medical Science Monitor. 25. 2032-2042. 10.12659/MSM.913375.

[13] Younus, H..Therapeutic potentials of superoxide dismutase, Int J Health Sci (Qassim). 2018: 12(3): 88–93.

[14] Vuori, Kristiina & Kanerva, Mirella. (2018). Superoxide dismutase (SOD) activity assay for zooplankton samples v1. 10.17504/protocols.io.mv7c69n.

[15] Berwal, Mukesh & Ram, Chet. (2018). Superoxide Dismutase: A Stable Biochemical Marker for Abiotic Stress Tolerance in Higher Plants. 10.5772/intechopen.82079.

[16] Kaushik, Rakesh & Dige, Mahesh & Dass, G. & Ramachandran, N. & Rout, Pramod. (2018). Superoxide Dismutase activity in response to heat stress in Jamunapari goats. Indian journal of biochemistry & biophysics. 55. 39-43.

[17] Gopal Mrsb, Rajesh & Elumalai, Sanniyasi. (2017). Industrial Production of Superoxide Dismutase (SOD): A Mini Review. Journal of Probiotics & Health. 05. 10.4172/2329-8901.1000179.

[18] Ighodaro, Osasenaga & Akinloye, Oluseyi. (2017). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine. 54. 10.1016/j.ajme.2017.09.001.

[19] Unlu, Ezgi & Takaç, Serpil. (2017). Improvement of superoxide dismutase activity using experimental design and radical promoters. Biotechnology & Biotechnological Equipment. 31. 1046-1054.

[20] Mukherjee, Monalisa & Rajput, Satyendra & Suhag, Deepa & Gupta, Apoorv & Singh, Aarti & Kumar, Vipin & Shakeel, Adeeba & Kumar, Arun & Nameet, Kaur. (2017). Therapeutic Implications of Superoxide Dismutase And Its Importance Inkinase Drug Discovery. Current Topics in Medicinal Chemistry. 17. 1-1. 10.2174/1568026617666170307112837.

[21] Deus, K.E. & Lanna, Anna & Abreu, F.R.M. & Silveira, R.D.D. & Pereira, Wendell & Brondani, Claudio & Vianello, Rosana. (2015). Molecular and biochemical characterization of superoxide dismutase (SOD) in upland rice under drought. 9. 744-753.