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Zoology

A Study of Toxic Effects of Nickel Chloride in *Rohul Labeo rohita*

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Abstract | **Original Research Article**

Labeo rohita is a member of the Cypriniformes and Cyprinidae families. It is also referred to as rui, rohu, and rohit. Thirty *Labeo rohita* fish, measuring twenty to twenty-five grams, were acquired for the current study from a fish seed farm in Head Balloki, Punjab, Pakistan. After applying the experiment samples were sent to laborteries for hematological and histopathological examination. The toxicological consequences of nickel chloride on the freshwater fish species *Labeo rohita* (Rohu) are examined in this study. The mean corpuscular hemoglobin (MCH) in the low and high-dose groups increased from 21.0 fL in the control group to 38.8 fL and 47.0 fL, respectively, according to hematological analysis. Additionally, there was variation in hemoglobin levels, which went from 8.06 g/dL in the control group to 6.3 g/dL in the low-dose group and then up to 8.5 g/dL in the high-dose group. White blood cell (WBC) counts showed a significant decline; the control group recorded 36.33 $\times 10^3/\mu L$, whereas the low and high-dose groups recorded 30.33 $\times 10^3/\mu L$ and 25.66 $\times 10^3/\mu L$, respectively. In contrast, the numbers of red blood cells (RBCs) rose in all experimental groups; in the low and high-dose groups, they were 1.66 $\times 10^6/\mu L$ and 1.3 $\times 10^6/\mu L$, respectively, compared to 1.14 $\times 10^6/\mu L$ in the control group. These results imply that *Labeo rohita* is exposed to nickel chloride, which causes notable physiological and cellular changes that may jeopardize the species' survival in contaminated aquatic settings. The study concentrated on hematological parameters and histological alterations in the gills after exposure to different nickel chloride concentrations. Significant gill injury was found, as evidenced by secondary lamellae shortening, hypertrophy, filament structure fusion, and gill epithelial hyperplasia. The findings emphasize how crucial it is to monitor and manage nickel pollution in aquatic environments to safeguard aquatic species that are essential to the survival and biodiversity of freshwater habitats, such as Labeo rohita.

Keywords: Histology, histopathology, Labeo rohita.

INTRODUCTION

Labeo rohita, also known as rui, rohu and rohit belongs to the families Cypriniformes and Cyprinidae (Sarma *et al.,* 2017). Cyprinid fish are members of the Labeo lineage, specifically the Cyprininae subfamily. The Labeos are limited to Africa and South-East Asia, whereas the Cyprininae are extensively distributed in Africa, Asia, Northern America, Guatemala, Southern Central America and Europe. With the Labeo lineage accounting for over 19.6% of all Asian cyprinid species composition, it is the second largest group of cyprinid fishes, after the Barbiinae. With more than 103 species worldwide, the genus Labeo (Cuiver, 1816) is without a doubt the most well-known grouping within the Cyprinidae family. There are 28 species in this genus, and they are found throughout South and Southeast Asia (Hasan *et al.,* 2007).

The most significant of the three Indian carp species utilized in carp polyculture systems is rohu (*Labeo rohita*). This elegant Indo-Gangetic riverine species inhabits the rivers of Bangladesh, Myanmar, and Pakistan in addition to the riverine systems of northern and central India. It was introduced to nearly every riverine system in India, including the Andaman Islands' freshwaters, where its population has been quickly expanding (Ayyappan & Jena, 2001). Numerous other nations, including the Philippines, Malaysia, Nepal, Sri Lanka, the former USSR, China, and certain African nations, have also accepted the species. This carp has a

centuries-old traditional culture that dates to the little ponds of eastern India (Ayyappan & Jena, 2003).

Early in life, rohu prefers the mainly rotifers and cladocerans that make up zooplankton, with phytoplankton serving as a fallback food supply (Bakhtiyar *et al.,* 2017). All zooplanktonic organisms as well as a few small phytoplankters like desmids, phytoflagellates, and algal spores are subject to strong selection during the fingerling stage. However, adults exhibit a strong positive selection for many phytoplankton. Rhu is basically a herbivorous organism column feeder that prefers algae and vegetation that is submerged in both its juvenile and adult phases. Moreover, the presence of sand, grit, and decomposing organic material in its stomach implies that it is a bottom-feeding animal (Saikia *et al.,* 2013). The fish can graze on delicate aquatic plants without being crushed or sewn shut because of its nibbling mouth style, which consists of soft lips with fringes, strong cutting edges, and no teeth in the bucco-pharyngeal area. The fish may filter water in order to feed on microscopic plankton, based on the modification of their short, hair-like gill rakers. Adults do not act in this manner; fingerlings and fry gather in ponds primarily to share food.

Rahu are eurythermal animals, meaning they cannot withstand temperatures below 14°C. Under typical cultivation conditions, this species grows quickly; in a single year, it can reach a maximum length of 35–45 cm and a maximum weight of 700–800 g. Its growth rate is typically higher in polyculture than in mrigal but lower in catla (Dwivedi & Nautiyal, 2012). The minimum age required for first maturity is two years for both sexes; it takes five years for females and four years for males (Majumder *et al.,* 2018). In the periphery and shallow areas of flooded rivers, spelling naturally happens. From April to September is rohu breeding season, which is usually linked to the south-west monsoon. When given a suitable diet, the species reaches maturity by the end of its second year in captivity. However, these lentic ponds' unfavorable reproductive environments necessitate artificial breeding. In addition to being polygamous, Rohu appears to be promiscuous. 22 to 31 °C is the ideal temperature range for spawning (Kaur *et al.,* 2018).

Figure 1.1: A view of *Labeo rohita* (Rrui, Rohit, or Rohu)

The body is quite elongated and bilaterally symmetrical, with a more arched dorsal profile than a ventral one. The eyes are dorsolaterally positioned and not visible from the exterior of the head. The head is scale-free. nose that is a little bit flattened and extends past the mouth without a lateral lobe; lips thick and edged with a distinct inner fold to each lip, either lobate or whole; mouth tiny and inferior; two tiny barbels hidden in a lateral groove on the maxilla; toothless jaws, an upper jaw that ends before the outer edge of the eye, and three rows of esophageal teeth Laterally, the pelvic fins and pupil are positioned; The dorsal fin is situated midway between the caudal fin's base and the tip of the nose, while the pectoral fin is apex-free. The lower lip is usually connected to the isthmus by a small or wide bridge, and the caudal fin is deeply forked. the pectoral and pelvic fins are placed laterally; There is no osseous spine on the pectoral fin; transversal lateral scale-rows between the pelvic fin base and the lateral line, six or six and a half; pre-dorsal scales, 12–16; lateral line scales, 40–44; absence of a lateral lobe and untruncated snout; distinct, complete lateral line that traces the median line of the caudal peduncle; color: silvery on the belly and flanks, blue on the back (Das *et al.,* 2012; Banerjee *et al.,* 2017; Shahid *et al.,* 2017).

Along with the two other significant carps in India, mrigal (*Cyprinus mrigala*) and catla (C*atla catla*), rohu is the primary species cultivated in carp polyculture systems (Ahmed *et al.,* 2012). The rohu usually has a longer feeding niche that extends from column to bottom than the other two species, which translates into higher stocking levels. The species is also raised in composite carp culture systems in India, which also house the two carp species from China, grass carp and silver carp as well as the three main carp species found there, common carp (*Cyprinus carpio*) (Malik *et al.,* 2020). The rohu percentage remains between 35 and 40 percent despite this six-species combination, which is similar to the three-species polyculture technique. Another outcome of the growing market demand and consumer desire for rohu in recent years is the two-species culture with catla. Approximately 100,000 hectares of ponds are utilized for the latter type of aquaculture in the Koleru Lake region of India, where rohu makes up more than 70% of the stock (Nissa *et al.,* 2022).

Carps are the most commonly farmed fish species outside of India. They are raised in countries such as Pakistan, Myanmar, Bangladesh, Vietnam, and Nepal. The most important of these carp species is the rohu. The silver, grass, and common carp are the three main species of carp grown in aquaculture in India. In each of these nations, these species are the most significant (Khan *et al.,* 2004). Primarily found in earthen ponds, rohu grow-out production is usually conducted in three-species polyculture systems alongside the other two major carp species in India. In certain instances, a six-species composite carp culture system may be employed, incorporating common, grass, and silver carp in different ratios, based on their inclinations towards distinct habitats and feeding niches (Sahu *et al.,* 2007). While output levels of 3-5 tonnes/ha/yr have recently been

achieved by scientific carp farming; these techniques are restricted to a small number of specialized regions. The majority of production still originates from intensive farming, which uses fertilizer and stocking as inputs to produce more modest production levels of 1-2 tonnes/ha/year (Rahman *et al.,* 2006). A combination of rice bran, wheat bran, and groundnut/mustard oil cake is used as supplemental feed. Fish health is monitored, weeds and predatory fish are controlled, fingerlings are stocked at a combined density of 4,000–10,000/ha (30–40 percent rohu), and ponds are fertilized with inorganic fertilizers and organic manures like cattle dung or poultry droppings (Paul & Giri, 2015). Throughout the year-long grow-out phase, rohu achieves a weight of 700–800 g. In some circumstances, farmers may occasionally harvest only a part of marketable size groups (>300 g). Over the course of 12 to 18 months in culture, rohu usually achieves a harvestable size of 1 to 1.5 kg. Production values of 6–8 tonnes/ha are recorded in these circumstances, with rohu making up about 70–80% of the biomass (Basavaraju & Varghese, 1980).

METHODOLOGY

Experimental Fishes

We purchased thirty *Labeo rohita* fishes, weighing between twenty and twenty-five grams, from a fish seed nursery unit in Head Balloki, Punjab, Pakistan. The fish were brought alive and housed in glass aquarium tanks with aerated, dechlorinated tap water for a week to acclimate them. The fish were acclimated to the laboratory environment using a recirculation aerated system with a water renewal system that changes the water every day to remove food remnants and fish faces. The fish were fed commercial fish pellets prior to the experimentation period of twenty days. Fish were allowed to fast for a day before being fed at a rate of 3.5% of their live body weight. The ideal conditions for *Labeo rohita* were maintained for the duration of the 20-day experiment: pH = 7.46±0.5, dissolved oxygen 7.25±0.23 mg/L, and water temperature 24.5±2.7°C.

Experimental Design

To make the stock solution, 5g of nickel chloride was weighed out and combined with 2 litters of distilled water. The water quality characteristics of the reservoir were established in the test media, according to APHA (Rendón-von Osten). After the fish had had time to acclimate, they were randomly divided into three equal groups. Each set of fish had ten fish randomly assigned to each 40-L tank. The first group served as a control group, receiving no chemical treatment and being housed in strictly controlled environments. Second group (low dosage) was administered a continuous 20-day dose of 280 ml of nickel chloride per 40 liters of water, whereas the third group (high dose) was administered a continuous 20-day dose of 560 ml of nickel chloride per 40 liters of water. The formulation's active ingredient, nickle chloride, provided the basis for figuring out the trial dosage. The similar water change procedure was used in the control group (Abou-Hadeed *et al.,* 2008).

Sampling

The fish were immersed in a 0.5 mL/L solution of 2-phenoxyethanol to put them to sleep at the conclusion of the experiment. For the micronucleus test, blood samples were taken from the caudal vein. The remaining volume was centrifuged for ten minutes at 904 g to extract serum for the liver function analysis.

Hematological study

Hematology, which literally means "the study of blood," is a field of medicine that looks at prognoses, the causes of illnesses, ways to prevent sickness, and how to cure blood system diseases. This procedure entails preventing illnesses that affect the creation of blood and its components, including blood cells, platelets, hemoglobin, blood arteries, and the coagulation process. Both human and animal blood are naturally occurring fluids that carry waste materials from the body's cells or outside of it, as well as essential elements like oxygen and nutrition to the body's cells. WBC (x 103/uL), RBC (x103/uL), neutrophil (%), lymphocytes (%), eosinocytes (%), and monocytes (%), Hb, Ht mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were all studied using the blood samples.

Tissue Collection

After being left out for a few days, fish will be gathered and carefully dried with a dry cloth. A fish net will be used to catch fish. Using a surgical blade to cut the belly skin from the ventral side, the fish will be dissected. The tissue from the liver and gills will be collected and placed in preservation tubes with formalin solution. A random selection of fish from each group will be used to dissect the fish and extract its tissues for histological examination.

Histology Study

The fish will be captured, and its heart, liver, kidney, and gills removed. The kidney, liver, heart, and gills will be removed from fish during their aseptic dissection and thereafter kept. Fish samples will be preserved in Bouni's solution. After being cleaned in 70% ethanol for a whole day, sections will be immersed in ethanol at several concentrations—80%, 90%, and 95%—for fifteen minutes each. After being immersed in xylene for twenty to thirty minutes, the sections will be replaced with a mixture of 50/50 paraffin and xylene and baked for two hours at 60°C.

After that, the samples will be replaced with soft paraffin for two hours at 60°C in an oven, followed by hand paraffin for another two hours at 60°C in an oven. After the specimen is obtained, it will be cut with a microtone measuring 2-3 mm. The slide containing Mayer's albumin will hold the specimen. The staining dyes that will be used are hematoxylen and eosin. After a little heating, one gramme of hematoxylen powder will dissolve in one liter of distilled water. To this solution,

five0.0 grams of sodium iodate will be added. The mixture will be heated in order to promote complete dissolution of the components. One gram of eosin powder will be dissolved in ten milliliters of ethyl alcohol. Next, to lessen the stain's visual impact, a concentrated 1/10 of a life of picric acid will be dissolved in the mixture. 80 cc of diluted water will be needed. After the stain production procedure is complete, a single drop of glacial acetic will be added. Slides will be soaked in water for staining after being de- paraphrased with xylene. The slides will be immersed in varying ethanol concentrations for those minutes: 100%, 95%, 80%, 70%, and 50%, in that order. The specimen will be stained with hematoxylen for one minute following a water rinse. Following that, it will be washed with water, submerged for three minutes in each of 95% and 100% ethanol, five minutes in xylene, and lastly stained with eosin. The slides will be air-dried using a blow dryer, soaked in xylene for five minutes, and then covered in cover slip and secured with Canada balsam. Finally, clove oil will be applied. The kidney, liver, heart, and gill slides from each treatment and control group will be viewed under a trinocular light microscope for histological analysis. A camera fixed on a standing platform will take photographs straight through the eyepiece's lens.

Statistical Analysis

The mean standard deviation of every data set is displayed. One-way analysis of variance (ANOVA) was performed using Mini Tab statistical software (version 20) to compare all parameters under study across four experimental settings.

RESULTS

Blood Physiology

Control group

In our experimental result the value of WBC in control group is from range of 35.16-37.5 ($\times10^{3/}$uL) with mean of 36.33, RBC ranges from 1.16-1.12 ($\times10^{3/}$uL) and their mean value is 1.14, Hemoglobin ranges from 7.2 - 9.10 (g/dl) and their mean value is 8.06 (g/dl), MCV rages from 1.7-2.1 with their mean values 1.9 (g/dl). In current research work the %age of HCT (PVC) ranges from 34.2-35.2 and their mean value is 34.7, MCH ranges from 19-23 % and their mean value is 21.0%, Platelets ranges from 50.1-55.5 % and their mean value is 52.8%, Neutrophil in range from 68-72 with mean of 70, %age of Lymphocytes in range from 24-28 with mean of 26, %age of monocytes in range from 1.8-2.2 with mean of 02 and the %age of Eosinophils is ranges from 1.6-2.4with mean value of 02 shown in table 4.1.

Table 4.1: Hematological values of control group

Parameters	Mean	SD
WBC ($\times10^{3/}$uL)	36.33	0.25
RBC ($\times10^{3/}$uL)	1.14	1.3
Hemoglobin g/dl	8.06	0.02
MCV%	1.9	1.6
HCT(PVC) %	34.7	0.14
MCH %	21.00	0.35
Platelets %	52.8	1.22
Neutrophils %	70	1.69
Lymphocytes %	26	0.66
Monocytes %	02	1.87
Eosinophils %	02	1.36

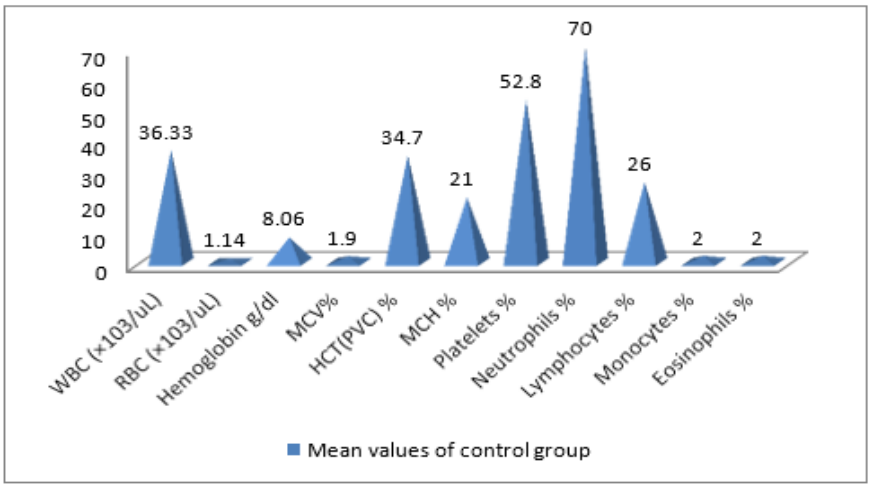

Figure 4.1: Graphical representation of hematological values of control group

Low dose group

In our experimental result the value of WBC in low group is from range of 28.2-32.34 ($\times10^{3}$/uL) with mean of 30.33, RBC ranges from 1.2-1.4 ($\times10^{3}$/uL) and their mean value is 1.3, Hemoglobin ranges from 5.9-6.7 (g/dl) and their mean value is 6.3 (g/dl), MCV rages from 1.6-1.10 % with their mean values 1.8 (g/dl). In current research work the %age of HCT (PVC) ranges from 24.0-27.0 and their mean value is 25.5, MCH ranges from 38.60-39.0 % and their mean value is 38.8 %, Platelets ranges from 37.90- 39.70 % and their mean value is 38.8%, Neutrophil in range from 57.0-63.0 with mean of 60, %age of Lymphocytes in range from 21.0-23.0 with mean of 22, %age of monocytes in range from 0.8-1.2with mean of 01 and the %age of Eosinophils is ranges from 0.9-1.1 with mean value of 01 shown in table 4.2.

Table 4.2: Hematological values of low group

Parameters	Mean	SD
WBC ($\times10^{3}$/uL)	30.33	0.65
RBC ($\times10^{3}$/uL)	1.3	1.24
Hemoglobin g/dl	6.3	0.36
MCV	1.8	0.24
HCT(PVC) %	25.5	1.33
MCH (fL)	38.80	1.02
Platelets	38.8	0.25
Neutrophils %	60	0.34
Lymphocytes %	22	1.88
Monocytes %	01	1.45
Eosinocytes %	01	1.36

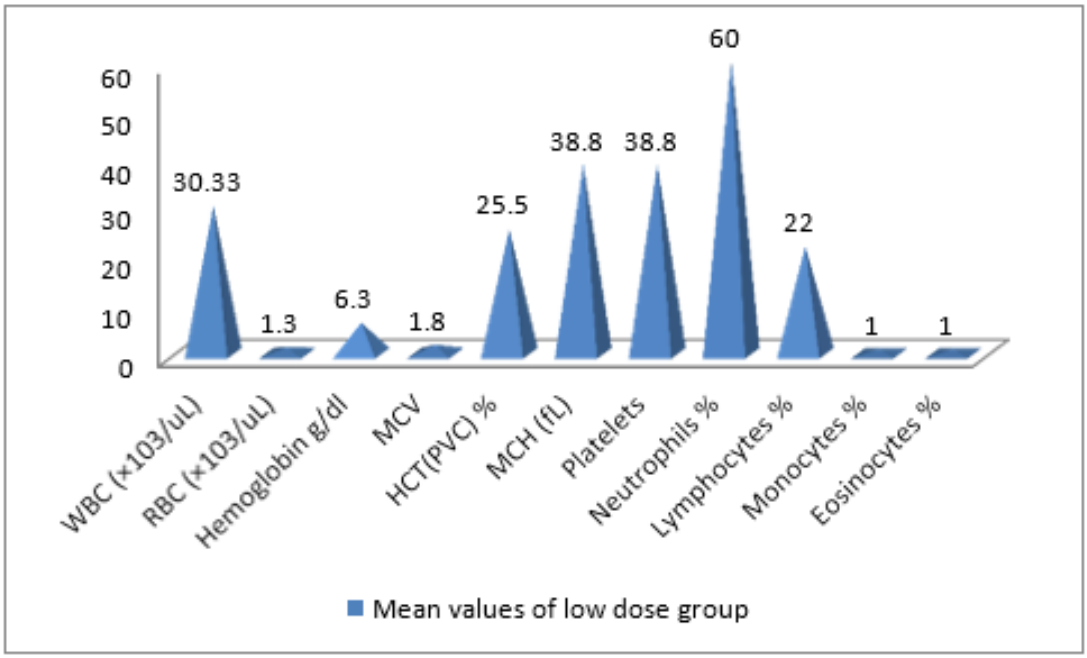

Figure 4.2: Graphical representation of hematological values of control group

Hematological values of high group

In our experimental result the value of WBC in high dose group is from range of 24.32-27.0 ($\times10^{3}$/uL) with mean of 25.66, RBC ranges from 1.5-1.82 ($\times10^{3}$/uL) and their mean value is 1.66, Hemoglobin ranges from 8.0-9.0 (g/dl) and their mean value is 8.5 (g/dl), MCV rages from 0.5-0.7 % with their mean values 0.6 (g/dl). In current research work the %age of HCT (PVC) ranges from 17.2-19.2and their mean value is 18.2, MCH ranges from 46.2-47.8(fL) and their mean value is 47.0(fL), Platelets ranges from 40.26-41.0 % and their mean value is 40.63 %, Neutrophil in range from 17.5-18.5 % with mean of 50, %age of Lymphocytes in range from 17.5-18.5 with mean of 18, %age of monocytes in range from 0.9-1.1 with mean of 01 and the %age of Eosinophils is ranges from 0.7-1.3 with mean value of 01 shown in table 4.3.

Table 4.3: Hematological values of High group

Parameters	Mean	SD
WBC (×10^{3}/uL)	25.66	0.68
RBC (×10^{3}/uL)	1.66	1.25
Hemoglobin g/dl	8.5	0.77
MCV	0.6	0.14
HCT(PVC) %	18.2	1.60
MCH (fL)	47.00	1.58
Platelets	40.63	0.34
Neutrophils %	50	1.25
Lymphocytes %	18	0.36
Monocytes %	01	0.89
Eosinophils %	01	1.23

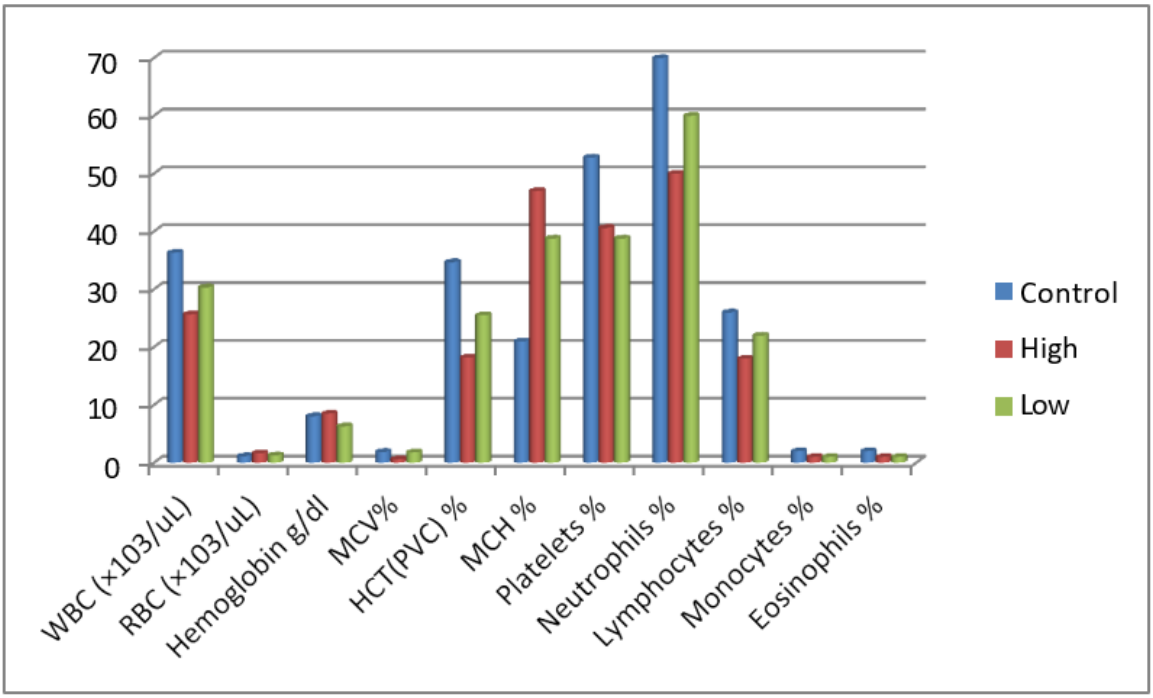

Figure 4.3: Graphical representation of hematological values of high group

Table 4.4: Shows comparison between control and experimental group

Parameters	Control	High	Low
WBC (×10^{3}/uL)	36.33	25.66	30.33
RBC (×10^{3}/uL)	1.14	1.66	1.3
Hemoglobin g/dl	8.06	8.5	6.3
MCV%	1.9	0.6	1.8
HCT(PVC) %	34.7	18.2	25.5
MCH %	21.00	47.00	38.80
Platelets %	52.8	40.63	38.8
Neutrophils %	70	50	60
Lymphocytes %	26	18	22
Monocytes %	02	01	01
Eosinophils %	02	01	01

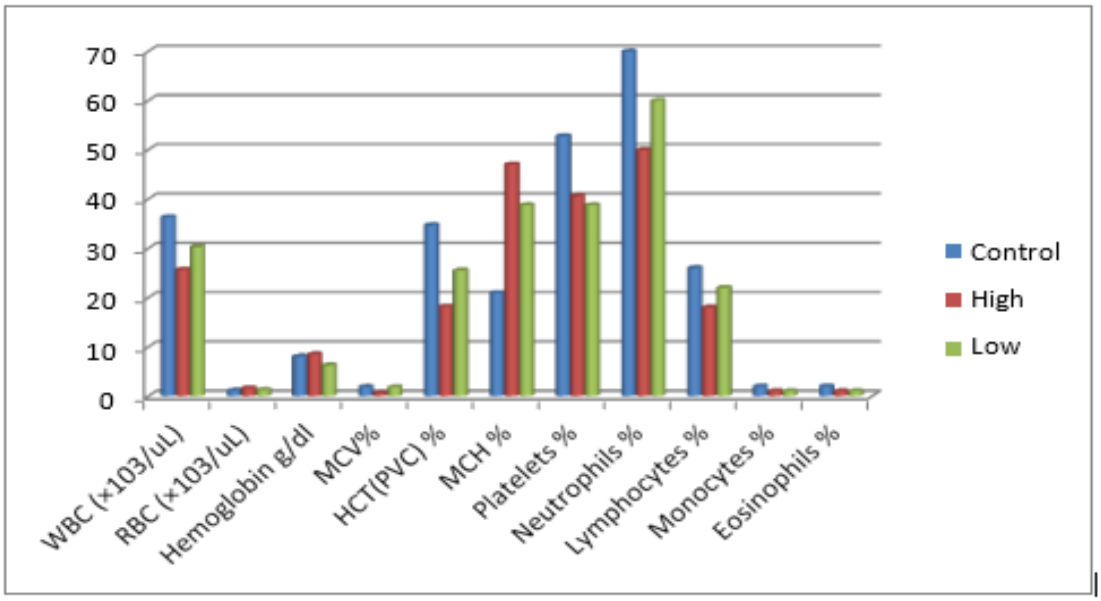

Figure 4.4: Shows graphical representation between control and experimental group

Histopathology of *Labeo rohita*

Histopathology of Liver

The control group's liver underwent histopathological examination, which showed normal hepatic architecture, normal-sized sinusoids, normal hepatocyte morphology, and normal porta hepatis figure 4.4 did not exhibit any signs of a granuloma, cancer, or inflammatory illness. On liver inspection, both treatment groups' sinusoids and central veins are noticeably enlarged. It was discovered that the liver cells had lost their polygonal form. Necrosis was observed in several areas. Splitting and vacuumolization also occurred in several areas. The injured cells' nuclei moved and took on an uneven form. Additionally, pyconotic and nuclear enlargement were noted. There is vacuolar degeneration in the hepatocytes show in figure 4.5 and 4.6.

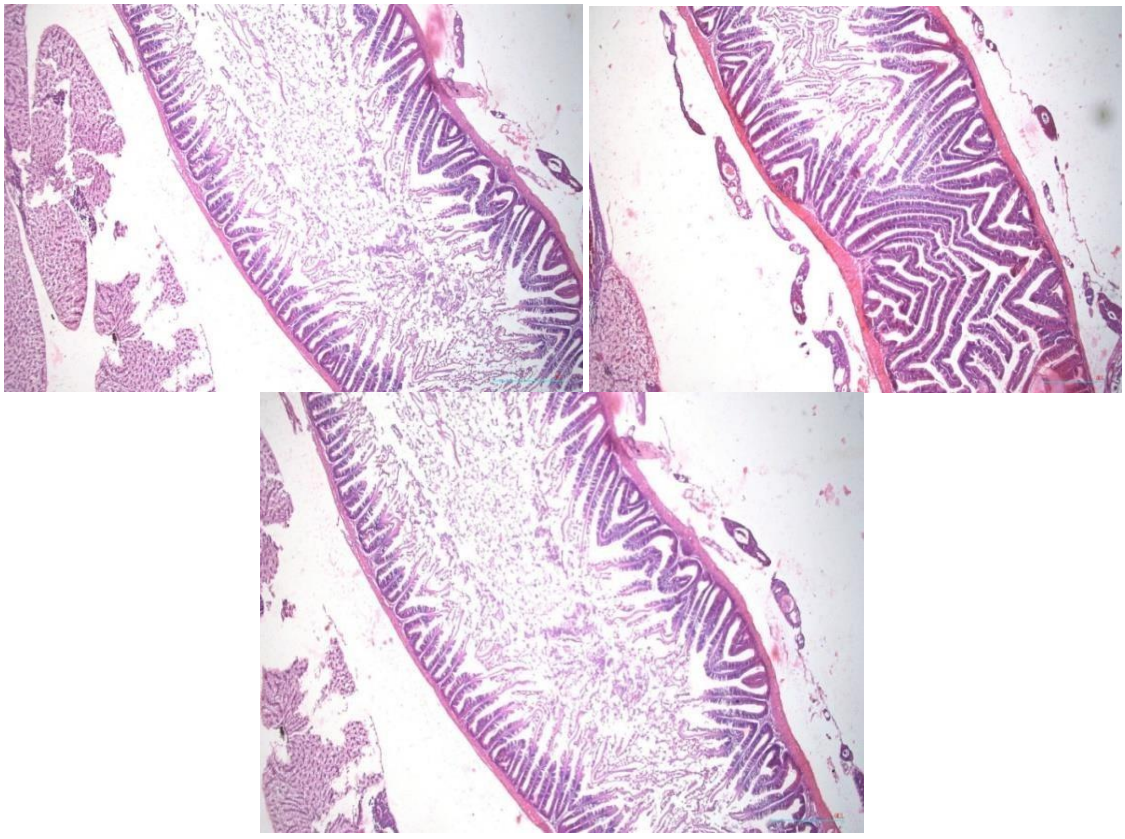

Figure 4.4: There is no sign of any cancer, granuloma, or inflammatory illness

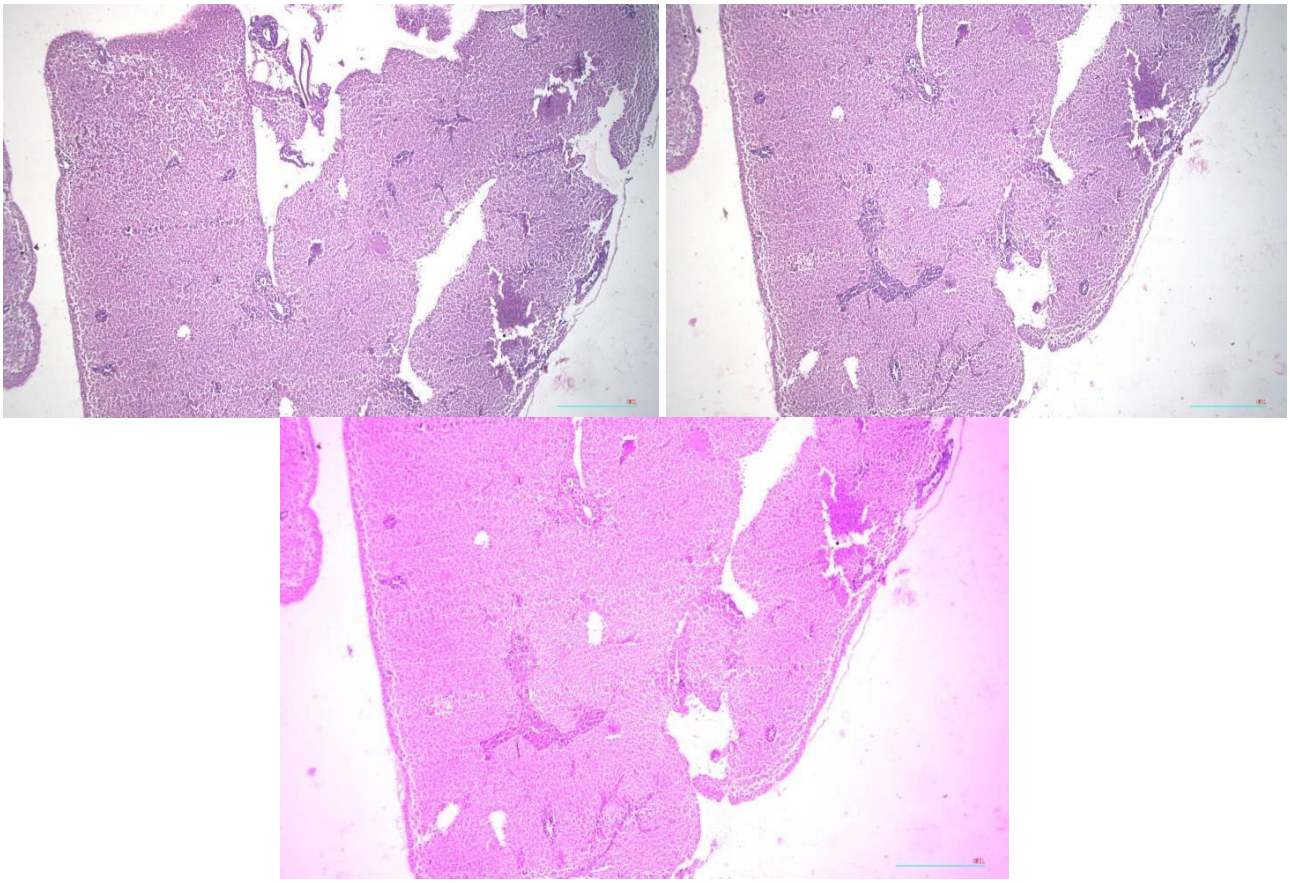

Figure 4.5: Upon liver inspection, both treatment groups' central veins and sinusoids are noticeably enlarged

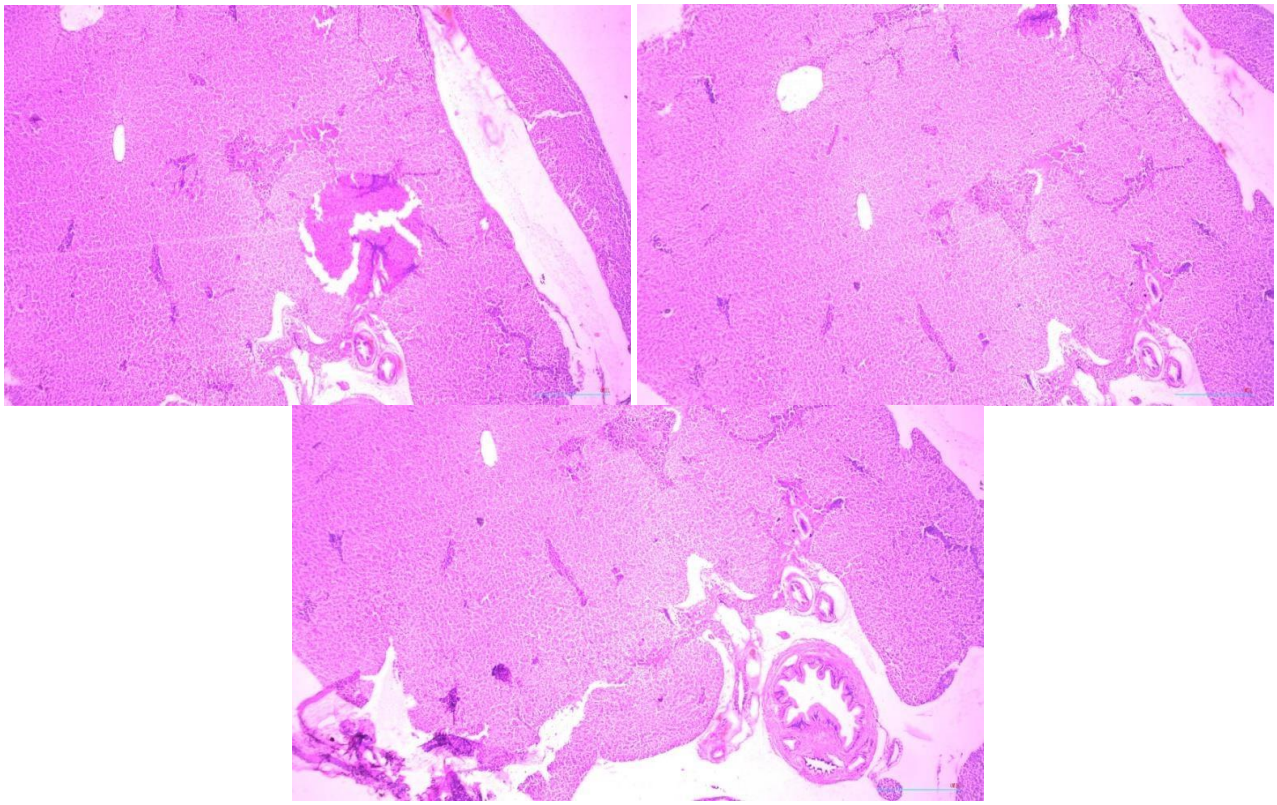

Figure 4.6: On liver inspection, both treatment groups' sinusoids and central veins are noticeably enlarged

Histology of Kidney

The control group's kidney histology analysis showed normal-looking renal tissue and nephrons. The ducts and tubules are operating normally. The ratio of the vasculature is constant. No indication of any cancer, granuloma, or inflammatory illness as depicted in Figure 4.7. Both treatment groups' kidney specimens had noticeably clogged arteries and localized hemorrhages. The hydropic degeneration of the renal tubular epithelium was observed. Oedematous glomerular Bowman's gaps indicate a lower glomerular filtration rate show in figure 4.8 and 4.9.

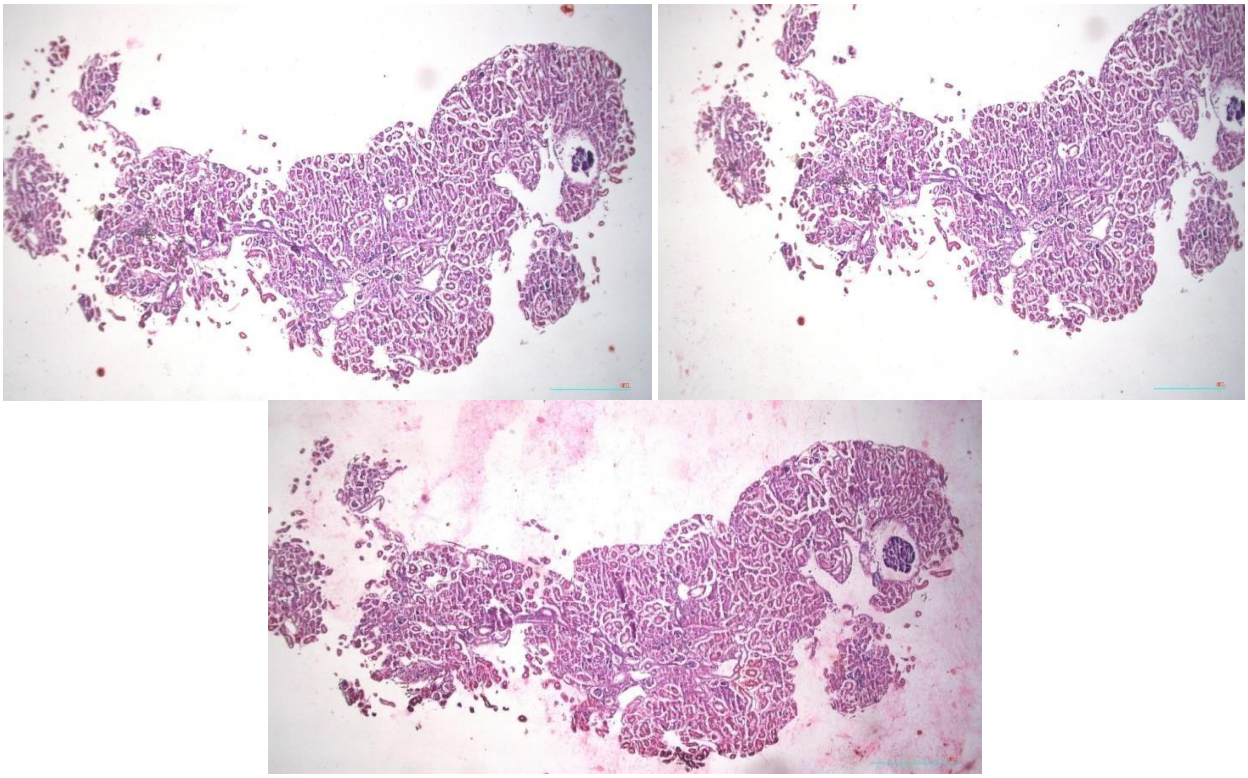

Figure 4.7: No indication of granuloma or distortion seen

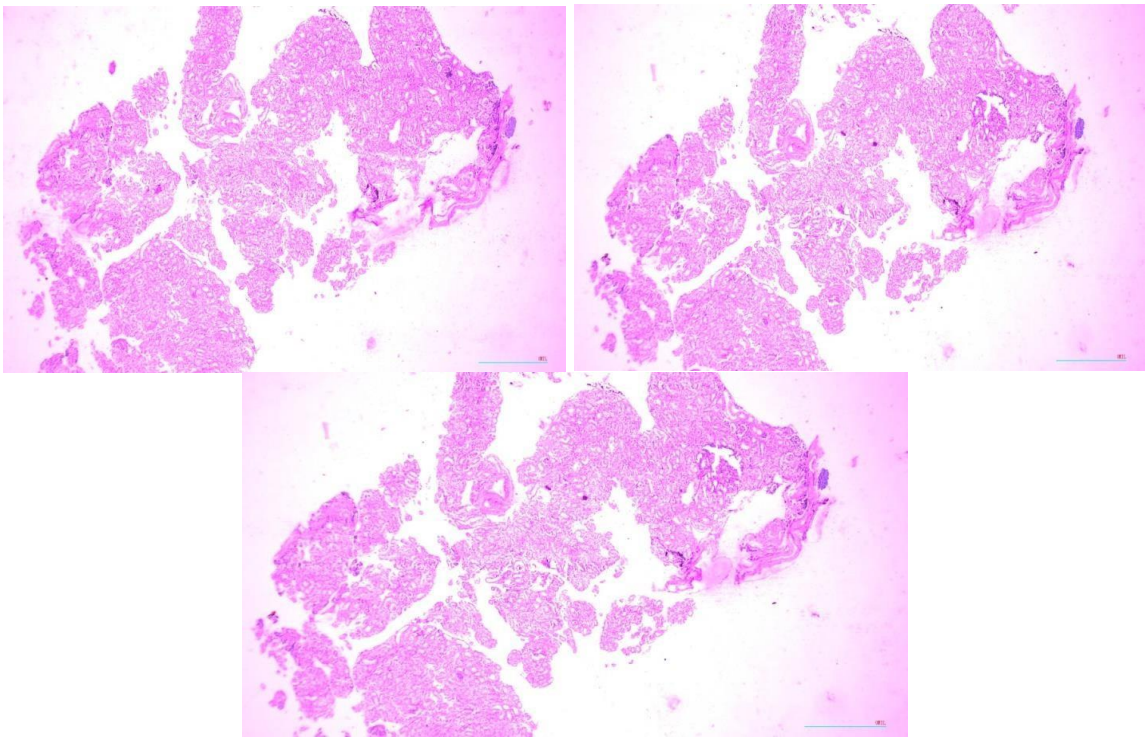

Figure 4.8: Oedematous glomerular Bowman's gaps indicate a lower glomerular filtration rate (GFR)

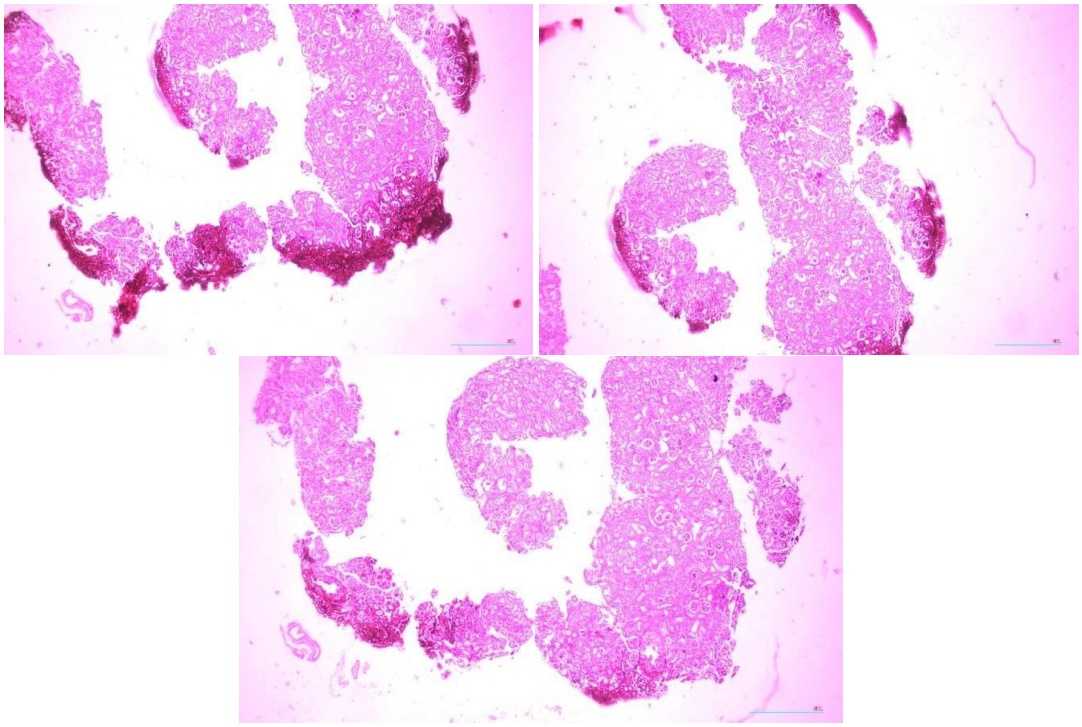

Figure 4.9: Hydropic degeneration of the renal tubular epithelium was observed

Histology of Gills

Gills histology group under control histological examinations on *Labeo rohita* revealed normal gills, as Figures 4.10. There was no sign of a tumor, granuloma, or inflammatory illness. The control sample exhibits normal structure of primary lamellae (PL) and secondary lamellae (SL). The treated group 1 and 2 gill specimens showed evidence of lamellar epithelium rupture and hemorrhages upon histological inspection. There are micro- aneurysms and congestion in the blood arteries. The exposed fish demonstrates normal arrangement of gill filament (GF) and secondary lamellae (SL); the exposed fish demonstrates hyperplasia at the ends of secondary lamellae (SL); the exposed fish demonstrates hyperplasia of secondary lamellae (SL); the exposed fish demonstrates hypertrophy and fusion at the ends of secondary lamellae (SL) and irregular interlamellar space; the exposed ish demonstrates fusion, hypertrophy, and necrosis of secondary lamellae show in figure 4.11 and 4.12.

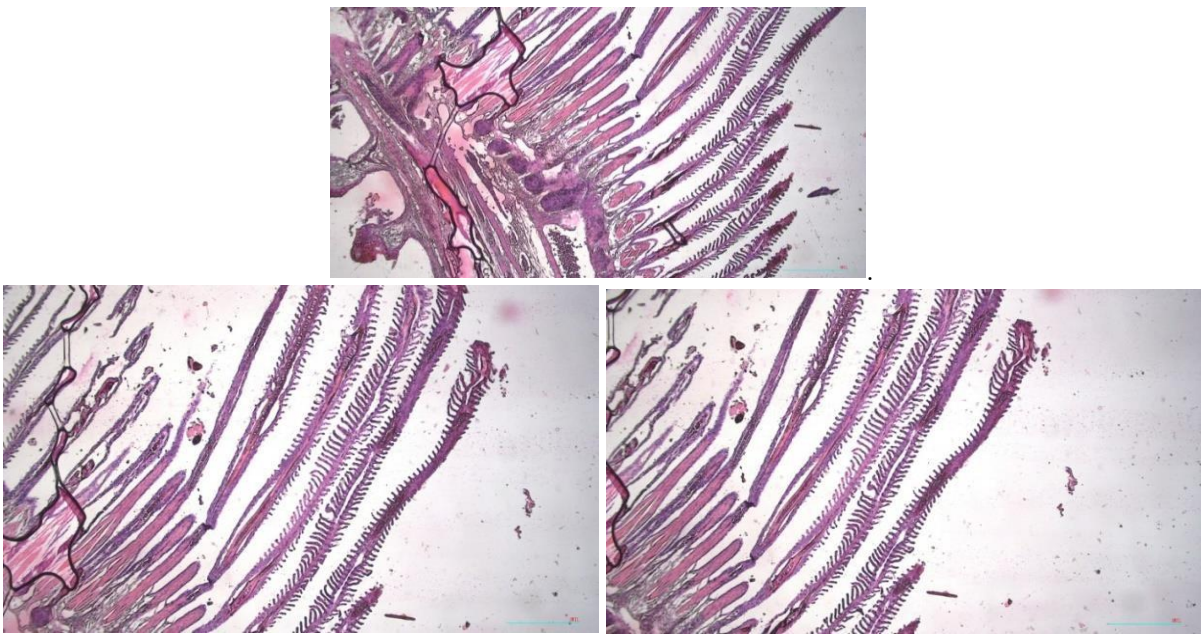

Figure 4.10: Histological Features of Control Gill: No granuloma, malignancy, or indication of an inflammatory illness was observed

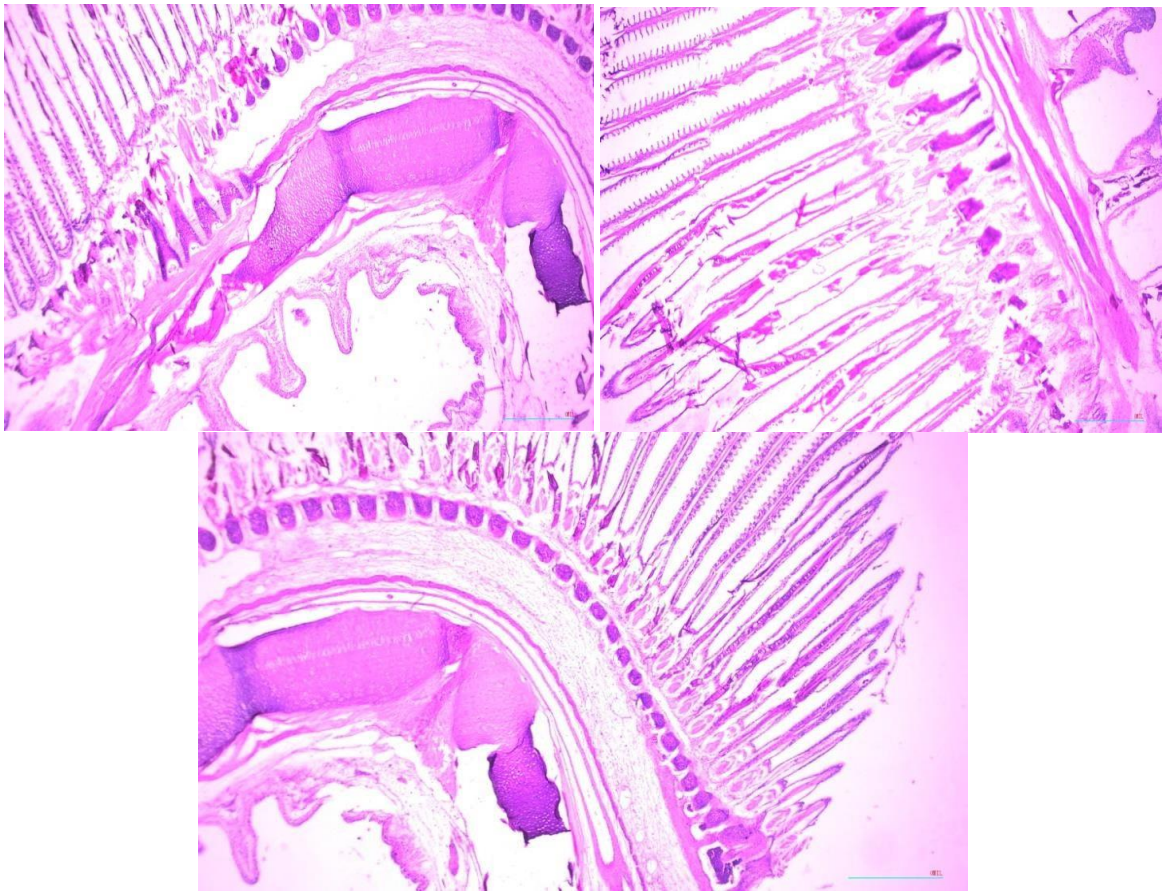

Figure 4.11: The low group gills samples underwent histological investigation, which showed lamellar epithelium tears and hemorrhages

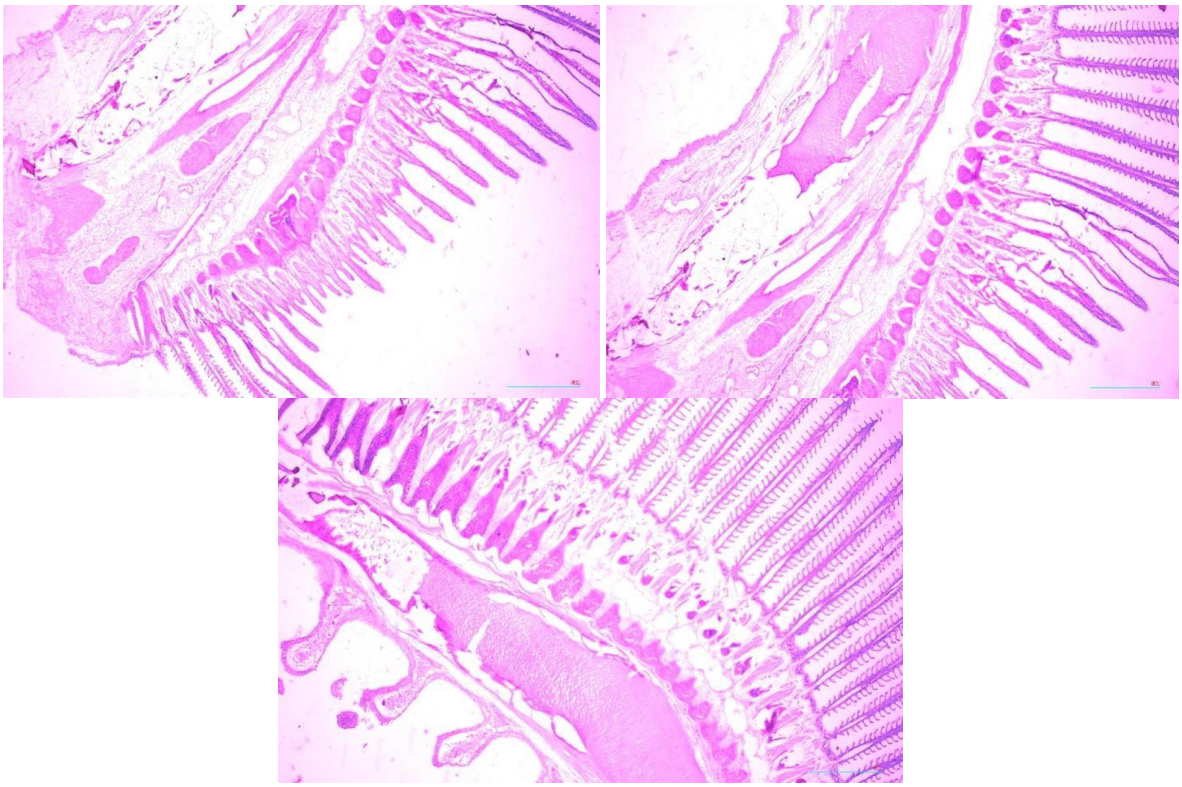

Figure 4.12: Histological examination of the low group gills specimen shows that the blood vessels are congested and exhibit micro-aneurysms

CONCLUSION

The study shows notable hematological and histological alterations in the freshwater fish *Labeo rohita,* highlighting the harmful effects of nickel chloride. The impairment of gill structures and changes in blood parameters including hemoglobin, MCH, WBC, and RBC levels highlight how susceptible aquatic life is to heavy metal contamination. The observed damage to the gills, which is characterized by morphological alterations including lamellae shortening and epithelial hyperplasia, points to a direct toxic effect of nickel chloride on respiratory function. Furthermore, the hematological alterations, specifically the decrease in white blood cell counts and the rise in red blood cell counts, are indicative of the fish's systemic stress reactions and possible coping strategies. The results highlight the importance of keeping an eye on and controlling nickel pollution in aquatic ecosystems to protect aquatic species like *Labeo rohita,* which are vital to the viability and biodiversity of freshwater habitats.

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