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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Evaluation of hemotoxic, nephrotoxic and hepatotoxic potential of phenol in *Labeo rohita*

## Fenolün *Labeo rohita* balığında hemotoksik, nefrotoksik ve hepatotoksik potansiyelinin değerlendirilmesi

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**Abstract:** The current study aimed to investigate the effects of phenol on the haematology, biochemical parameters, and histopathology of *Labeo rohita* in the laboratory conditions. Three groups were maintained as control, low dose (0.30 mg/L) and high dose (0.40 mg /L). The results after 28 days exposure to phenols showed changes in hematological parameters as RBCs, WBC, PLT, HGB, MCH, MCHC, RDW and PCT significant decrease and HCT, MCV, RDW-SD, MPV, and PDW significant decrease as compared with control group. Similarly, phenol exposure showed significant alterations in biochemical parameters as decrease the level of cholesterol, triglycerides, HDL, blood glucose, total protein, TSH, albumin and globulin while increase the level of ALT, AST, T3, T4, urea and creatinine. Histological observations showed the damage caused by phenol in kidney tissues was sinusoidal spaces, elongated tubules, cluster nuclei formation, damages parenchyma and melano-macrophages. Histology of liver showed pyknotic nuclei formation, eosinophilic granules in cytoplasm, cytoplasmic vacuolation and necrosis. In gills, curved gills lamellae, oedema, lamellar aneurysm, lamellar disorganization, bone cell deformities and hyperplasia of epithelial cells were observed. Histology of muscles also showed pigmented cells around blood cells, necrosis, intermyofibrillar, oedema and atrophy in muscle tissues. The overall results showed that phenols are highly toxic to aquatic species.

**Keywords:** Aquatic toxicity, phenols, hepatotoxicity, nephrotoxicity, fish, biochemistry

**Öz:** Bu çalışmanın amacı, laboratuvar koşullarında *Labeo rohita* balığının hematolojisi, biyokimyasal parametreleri ve histopatolojisi üzerindeki fenol etkilerini araştırmaktır. Kontrol, düşük doz (0,30 mg/L) ve yüksek doz (0,40 mg/L) olmak üzere üç grup oluşturulmuştur. 28 günlük fenol maruziyetinden sonra elde edilen sonuçlar, kontrol grubuyla karşılaştırıldığında, hematolojik parametrelerde RBC, WBC, PLT, HGB, MCH, MCHC, RDW ve PCT'de anlamlı azalma ve HCT, MCV, RDW-SD, MPV ve PDW'de anlamlı azalma olduğunu göstermiştir. Benzer şekilde, fenol maruziyeti, kolesterol, trigliserit, HDL, kan şekeri, toplam protein, TSH, albümin ve globulin seviyelerinde azalma, ALT, AST, T3, T4, üre ve kreatinin seviyelerinde ise artış gibi biyokimyasal parametrelerde önemli değişiklikler göstermiştir. Histolojik gözlemler, böbrek dokularında fenolün neden olduğu hasarın sinüzoidal boşluklar, uzamış tübüller, küme çekirdek oluşumu, parankima hasarı ve melano-makrofajlar olduğunu göstermiştir. Karaciğer histolojisi, piknotik çekirdek oluşumu, sitoplazmada eozinofilik granüller, sitoplazmik vakuolasyon ve nekroz göstermiştir.Solungaçlarda eğri solungaç lamelleri, ödem, lameller anevrizma, lameller düzensizlik, kemik hücresi deformiteleri ve epitel hücrelerinin hiperplazisi gözlenmiştir. Kasların histolojisi ayrıca kan hücreleri çevresinde pigmentli hücreler, nekroz, intermiyofibriller ödem ve kas dokularında atrofi göstermiştir. Genel sonuçlar, fenollerin sucul türler için oldukça toksik olduğunu ortaya koymuştur.

**Anahtar kelimeler:** Su toksisitesi, fenoller, hepatotoksisite, nefrotoksisite, balık, biyokimya

## INTRODUCTION

Aquaculture is rapidly growing field of food production due to increasing demand for aquatic food for increasing population of the world (Subasinghe et al., 2009). The amount of capture fisheries has reached its full capacity and world is switching modern aquaculture to close the gap between the supply and demand of aquatic food (Li et al., 2011). The fish species is highly nutritive and contain Omega-3 fatty acids, Vitamin A, Vitamin B, Vitamin C and Vitamin D. Vitamin D is only found in a few foods in this way eating fish will protect you from osteoporosis (Haq et al., 2014). South Asian rivers are habitat for the rohu (*Labeo rohita*), a member of carp family. Habitat ranges from India, Southeast Asia, Pakistan, Bangladesh, Nepal, Myanmar and Vietnam. It is also introduced to few rivers in Sri Lanka and Peninsula India (Agri-farming, 2018). Rohu is widely cultivated in farms and introduce in various rivers due to its taste and market demands.

Phenols are being used as organic compound in colours, textiles, medicines, and other products (Muthukumaravel et al., 2021). The other industries might be coke manufacturers, wood products, slate, oil refineries, aniline paint (Maistrenko and Klyuev, 2004; Michałowicz and Duda, 2007), dyeing, pulp mills, petrochemicals, textiles, resin, leather, and coal refineries (Ying et al., 2002). They are xenobiotics that cause stress (Afandi et al., 2019). Numerous chemicals have been dumped into natural environments as a result of home and industrial waste (Hori et al., 2006). Several industrial wastewaters and generic pesticides contain phenol and its derivatives which frequently accumulate in the freshwater and marine habitats (Gupta et al., 1983). Natural phenomena such as degradation of plant matter (Ibrahem, 2012), metabolizing aquatic organisms, and the transformation of organic materials in water, sediment and the metabolic breakdown are also

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responsible for phenol in the aquatic environment (Ali et al., 2011; Michałowicz and Duda, 2007). It is benzene's hazardous metabolite (Medinsky et al., 1994). It accumulates in the aquatic food chain due to its lipophilic nature and become dangerous to both the environment and organism. The Environmental Protection Agency of the United States listed phenol as one of the first substances to be classified as toxic and especially as an ecotoxin (Remya et al., 2014).

Phenol impacts the physiology of fishes in the aquatic ecosystem. It can alter the enzymatic, hormonal, haematological and reproductive systems. The impact of the phenol on the fish reproductive systems are documented in a variety of ways, such as decreased plasma sex hormone, masculinization, increased fecundity, altered secondary sex traits, smaller gonads, delayed maturation, and decreased vitellogenin in females (Keya et al., 2018). It also increases the interfollicular gaps as a result of lower gonadal oocyte average diameter and mature females' reduced Gonadosomatic index (Keya et al., 2018). Some fish treated with phenol displayed altered metabolism of protein and carbohydrate, weight loss and sterility (Avilez et al., 2008). It interferes with the kidney detoxification mechanisms, increasing accumulation in the kidney and reducing the elimination of phenol (Sannadurgappa et al., 2007). Phenol quickly penetrates and total carbohydrates decrease in muscle and liver while increasing in kidneys (Remya et al., 2014). It increases the level of LDA and ALT in liver, muscle, gills and kidney. The level of serum cholesterol decreases (Zaki et al., 2011), phospholipid composition of the plasma membrane alters (Kotkat et al., 1999), and immunological system of fish become compromised (Dunier and Siwicki, 1993). It also affects the feeding rate when subjected to sub-lethal concentration (Sannadurgappa et al., 2007). Phenol causes histopathological changes in skin, liver, spleen and heart (Mitrovic et al., 1968) and damage to erythrocytes (Halsband and Halsband, 1963). In the phenolic-treated fish, the intestine shows intestinal epithelium tissue damage, inflammation, and binding of neighbouring cuboidal epithelium-made villi (Muthukumaravel et al., 2021). Phenols harm the eyes, kidneys and muscles, in addition to disrupting metabolism (Holmberg et al., 1972), liver dysfunction (Inyang et al., 2019) and neurotoxicity (Kaila, 1982). In the brain degenerative alterations, cellular edema, localized gliosis and nerve cell necrosis with neuronophagia were recorded (Ibrahem, 2012).

Fish is an important source of food undergoes exposed to contaminated water and act as indicator to evaluate the toxicity of aquatic environment. The contamination in water badly affects the health of fish (Saleem et al., 2022). Fish is prominent member of aquatic food chain, therefore, any abnormality in fish leads to cause alteration in human. To assess the toxicity of phenol we used *Labeo rohita* in the experimentation.

## MATERIALS AND METHODS

### Settings of experiment

Model species *Labeo rohita* was purchased from government of Punjab fish hatchery, Manga mandi, Lahore, Punjab, Pakistan and brought to fisheries Laboratory at zoology department, University of Okara, Renala Khurd, Okara, Punjab, Pakistan. Fish first acclimatized with $KMnO_4$ for 7 days. The fish were transferred to aquarium (40 litre) made of glass with tap water having recirculation aerated system along with water renewal system to remove food waste and faeces of fish by changed water. Water physicochemical parameters such as pH 7.46 ± 0.26 levels, temperature 24.5 ± 2.7 °C and dissolved oxygen 7.25 ± 0.23 mg/L in the exposure media were routinely monitored with pH meter (Lutron PH-223 Pen), thermometer (Beurer FT 13), and DO meter (JPB-70A), respectively.

The fishes were feed with commercial fish diet at morning hours.

### Chemical

Pure grade of phenol ($C_6H_6O$) (≥99.5% purity) (crystalline form) was purchased from Sigma-Aldrich (USA) (Catalog No. P1037) with a freezing point of 39.5– 41.0 °C (Gad and Saad, 2008). Stock solutions were prepared using deionized water and stored in glass container at 4°C.

### Experimental design and treatment

For the experimental trial, three groups were made: a control group and two treatment groups (Low and High) each with a triplicate. 15 fish were placed in each group. Fish in low and high groups were exposed to 0.30 mg/L ($1/20^{th}$) and 0.40 mg/L ($1/15^{th}$) to Phenol for 28 days. The doses were selected after evaluating $LC_{50}$ value (6 mg/L) for 96-h by following probit analysis method (Finney, 1971). Every second day, 90% of the water in each glass aquarium is replaced with freshwater.

### Hematobiochemical analysis

After 28-day exposure to phenol, blood was collected from caudal vein via BD syringe and stored in EDTA tubes for hematological analysis and in gel vials for biochemical analysis. Automated Haemocytometer was used for Haematological analysis. Blood analysis includes platelets count, WBCs counts, Lymphocytes, Granulocytes, Haemoglobin concentration, MCH, MCHC, RBC, MCV, HCT, RDW, MPV, and PCT. Serum was separated by centrifugation at 3500 rpm for 12 minutes at 4 °C. Automatic Chemistry Analyzer (Hitachi 7600) was used for biochemical analysis. Hormonal levels in fish were determined using competitive ELISA kits, according to the approach of Seibel et al. (2021). Biochemical parameters are measured using colorimetric or enzymatic-kinetic techniques with commercial diagnostic kits (Sigma Aldrich).

### Histology

The gills, kidney, muscle and liver tissues were sampled on 28th day and fixed in 10% formalin solution. Fixation was done by series of dehydration steps. First, tissues were placed in 80% ethanol, then 90%, and finally in 100% ethanol for a period of 2 h in each dilution. The tissues were then placed in cedar wood oil until clear. The clear tissues were placed in paraffin wax for 30 min at 60 °C in the incubator. The paraffin wax was

changed after 30 min and tissues again placed in an incubator for 12 h at 60 °C. The paraffin wax was third time changed and placed in an incubator for 12 h at 60 °C. The box blocks of each tissue were made and mounted into plastic casters. The embedded tissue was fixed in rotatory microtone and 3–5-µm-thick sections were cut for each tissue. Each section was transferred to a clean slide and stretched on Fisher slides, warmed and remained it on slide for 24 h (S. Ahmad et al., 2025). Sections of micrometer were photographed under the microscope at 40X (Olympus CX23).

### Statistical analysis

Data was analysed through GraphPad Prism (V9.3.1.4). One-way ANOVA test followed by Dunnett's multiple comparison post hoc test applied to compare experimental groups with control at $p < 0.05$ level of significance.

## RESULTS

### Hematological analysis

Results of haematology of *L. rohita* revealed in Table 1. Blood HCT, MCV, RDW-SD (%), MPV and PDW (%) were significantly increased as compare to control. Same was in the case of neutrophils, and eosinocytes. All other blood parameters such as RBC, WBC, platelets count, HGB, MCH, MCHC, RDW and PCT were significantly decreased. Monocytes and lymphocytes do not show any significant change after exposure to phenol for 28 days.

**Table 1.** Hematological parameters of *L. rohita* after 28 days exposure to phenol. The asterisk (*) represent the significant ($p < 0.05$) values.

| Parameters | Control | Low (0.30 mg/L) | High (0.40 mg/L) |
|---|---|---|---|
| HGB (g/dl) | 5.6±0.1 | 3.8±0.1* | 2.5±0.1* |
| WBC (x10$^3$/µL) | 15.2±0.49 | 4.2±0.15* | 3.31±0.02* |
| RBC (x10$^6$/ µL) | 1.18±0.02 | 0.9±0.12* | 0.5±0.14* |
| HCT (%) | 4.9±0.1 | 9.0±0.14* | 7.0±0.16* |
| MCV (FL) | 41.6±0.37 | 109.0±0.16* | 112.0±0.11* |
| MCH (pg) | 47.0±0.26 | 35.0±0.11* | 36.0±0.12* |
| MCHC (g/dl) | 113.0±0.49 | 39.0±0.15* | 42.0±0.15* |
| RDW | 23.7±1.01 | 17.8±0.13* | 16.0±0.13* |
| RDW-SD (%) | 32.6±0.89 | 54.0±0.11* | 59.0±0.15* |
| PLT (x10$^3$/ µL) | 203.0±1.06 | 133.0±0.60* | 29.0±0.14* |
| MPV (fl) | 5.4±0.26 | 9.8±0.14* | 6.4±0.1* |
| PDW (%) | 15.2±0.37 | 19.2±0.12* | 17.3±0.12* |
| PCT (%) | 0.1±0.05 | 0.13±0.01 | 0.08±0.01* |
| Neutrophils (%) | 70.0±0.60 | 70.0±0.13 | 72.0±0.17* |
| Lymphocytes (%) | 26.0±0.37 | 25.0±0.15 | 25.0±0.15 |
| Monocytes (%) | 2.0±0.05 | 3.0±0.1* | 2.0±0.16 |
| Eosinophils (%) | 2.0±0.05 | 2.0±0.1 | 1.0±0.12* |

### Biochemical parameters

Biochemical parameters of *L. rohita* exposed to phenol showed in Figure 1 including Cholesterol, Triglycerides, LDL, HDL and VLDL decrease in treated groups**.** ALT and AST enzymes increase in phenol exposure groups than in control revealed in Figures 2(a)(b). Blood glucose level was significantly decrease possibly due to down regulation of blood glucose regulating hormones or enhanced energy demand showed in Figure 3(d). T3 and T4 level in control was low but in low dose and high dose increases due to increase of energy demand to compensate the stress caused by phenol in Figures 4(a)(b). Figure 4(c) showed TSH level decrease in treated groups. Urea level also significantly increases compared to control showed in Figure 5(a). revealed in Figure 5(b) Blood urea nitrogen also fluctuated when fishes exposed to low and high dose. Creatinine showed alleviation in low dose and high dose in Figure 5(c).

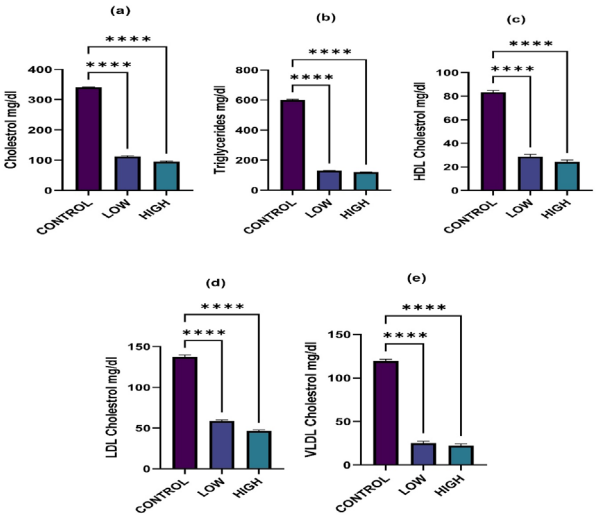


**Figure 1.** Comparison between control, low dose (0.30 mg/L) and high dose (0.40 mg/L) effects of phenol on lipid profiles of *L.rohita*

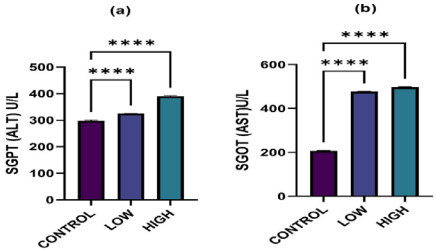


**Figure 2.** Comparison between control, low dose (0.30 mg/L) and high dose (0.40 mg/L) effects of phenol on liver enzymes of *L. rohita*

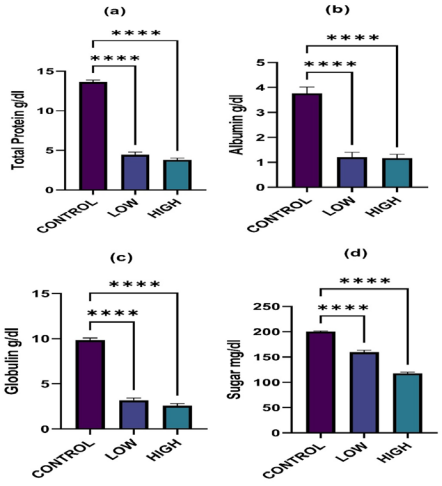


**Figure 3.** Comparison between control, low dose (0.30 mg/L) and high dose (0.40 mg/L) effects of phenol on the level of serum proteins and glucose of *L. rohita*

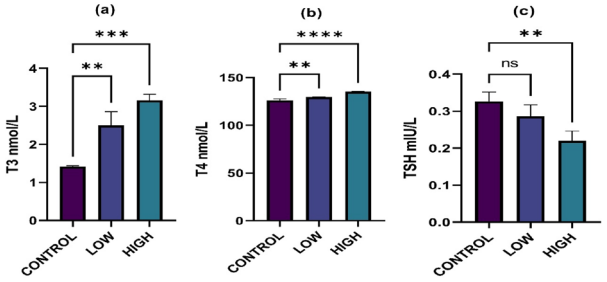


**Figure 4.** Comparison between control, low dose (0.30 mg/L) and high dose (0.40 mg/L) effects of phenol on the level of thyroid hormones of *L. rohita*

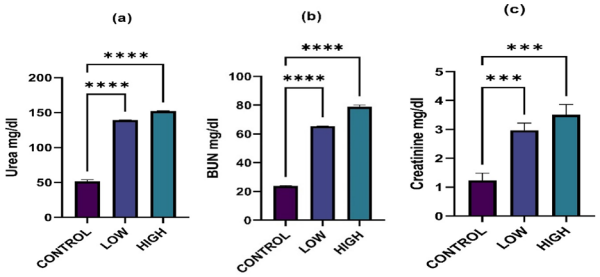


**Figure 5.** Comparison between control, low dose (0.30 mg/L) and high dose (0.40 mg/L) effects of phenol on the kidney functioning parameters of *L. rohita*

### Histological studies

Liver tissues after treated with phenol undergoes pyknotic nuclei, eosinophilic granules in cytoplasm, cytoplasmic vacuolation and necrosis. More severe vacuolation and necrosis of hepatic cell treated with high dose of phenol (0.40 mg/L) shows in Figure 6. Kidney tissue having sinusoidal spaces, elongated tubules, cluster nuclei formation, damages parenchyma and melano-macrophages in low dose but severe in high dose revealed in Figure 7. Figure 8 shows that gills highly damaged by phenol exposure leads to curved gills lamellae, oedema, lamellar aneurysm, lamellar disorganization, bone cell deformities and hyperplasia of epithelial cell. Further shows in Figure 9 that pigmented cells around blood cells, necrosis, intermyofibrillar, oedema and atrophy in muscle tissues.

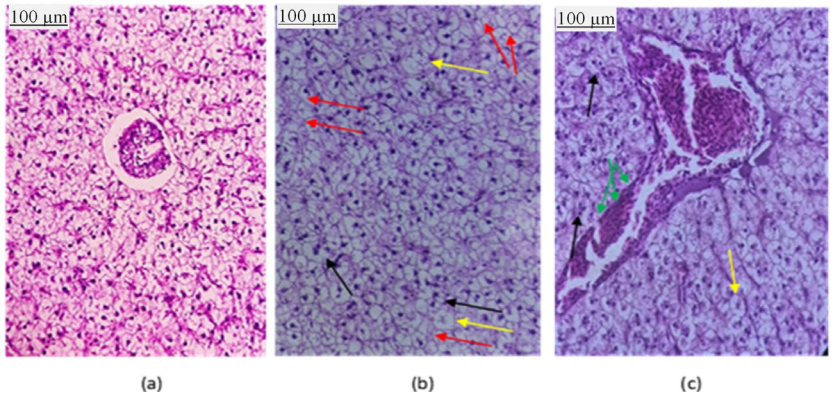


**Figure 6.** Histology of liver tissues of *Labeo rohita* at 40X. a) control shows normal tissues. b) low dose (0.30 mg/L) shows hepatic tissues damage (Red arrows indicate cytoplasmic vacuolation, yellow arrows represent eosinophilic granules in cytoplasm, and black arrows point to pyknotic nuclei). and high dose (0.40 mg/L) shows different histological anomalies in hepatic tissues (Yellow arrows represent eosinophilic granules in cytoplasm, black arrows point to pyknotic nuclei and green arrows indicate focal necrosis).

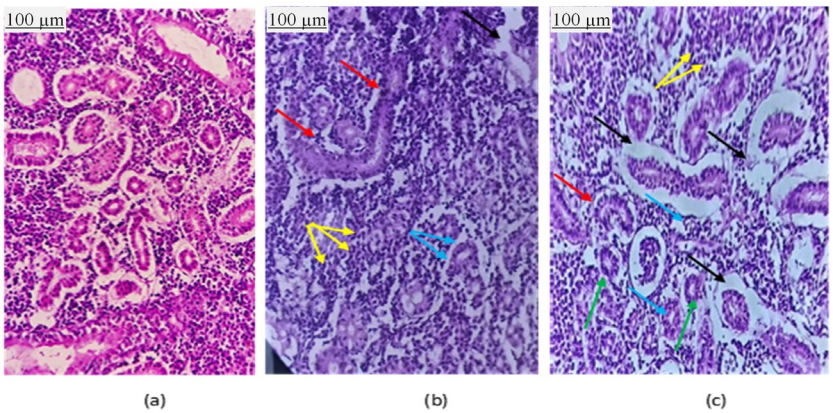


**Figure 7.** Histology of kidney tissues of *Labeo rohita* at 40X. a) control shows normal tissues. b) low dose (0.30 mg/L) shows kidney tissues damage (Red arrows indicate elongated tubules, yellow arrows represent cluster nuclei formation, black arrows point to sinusoidal spaces, and blue arrows indicate damaged parenchyma) and high dose (0.40 mg/L) shows different histological anomalies in kidney tissues (Red arrows indicate elongated tubules, yellow arrows represent cluster nuclei formation, black arrows point to sinusoidal spaces, blue arrows indicate damaged parenchyma, and green arrows represent melano-macrophages).

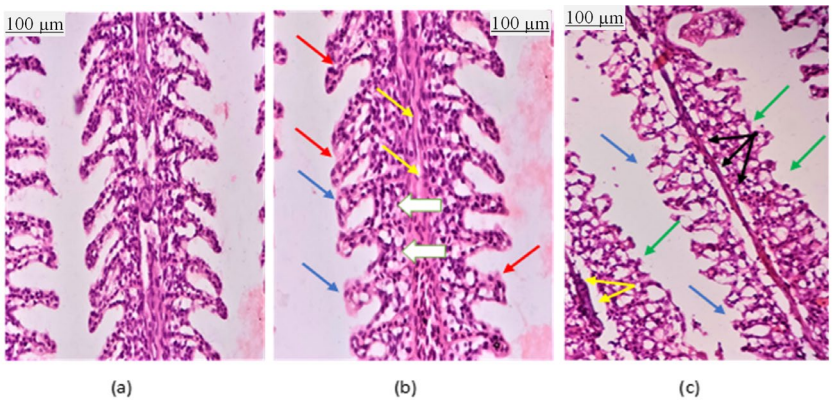


**Figure 8.** Histology of gill tissues of *Labeo rohita* at 40X. a) control shows normal tissues. b) low dose (0.30 mg/L) shows gill tissues damage (Red arrows indicate lamellar aneurysm, yellow arrows represent oedema, white arrows point to hyperplasia of epithelial cell, and blue arrows indicate curved gills lamellae) and high dose (0.40 mg/L) shows different histological anomalies in gill tissues (Green arrows indicate lamellar disorganization, yellow arrows represent oedema, black arrows point to bone cell deformities, and blue arrows indicate curved gills lamellae).

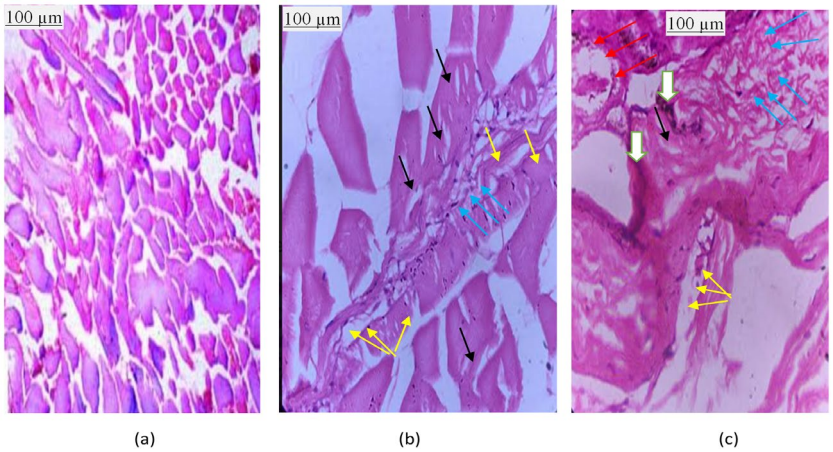


**Figure 9.** Histology of muscle tissues of *Labeo rohita* at 40X. a) control shows normal tissues. b) low dose (0.30 mg/L) shows muscle tissues damage (Blue arrows indicate atrophy, yellow arrows represent intermyofibrillar, and black arrows point to necrosis) and high dose (0.40 mg/L) shows different histological anomalies in muscle tissues (Blue arrows indicate atrophy, yellow arrows represent intermyofibrillar, black arrows point to necrosis, white arrows indicate pigmented cells around blood cells, and red arrows represent oedema).

## DISCUSSION

The human and their environment are being exposed to large number of chemical every year which causes toxicity on environment (Mahmood et al., 2023). When these chemicals added in water become part of that environment and causes contamination in water bodies. The present study was conducted to find out the effect of phenol on haematology, histology, biochemical parameters and activity of two hormones T3 and T4 in *L. rohita* which is a freshwater fish.

The results of hematological parameters showed that Blood HCT, MCV, RDW-SD, MPV and PDW were increased significantly. Similar findings were observed by McKim et al. (1970) and . All other blood parameters like RBC, WBC, platelets count, HGB, MCH, MCHC and PCT were significantly decreased. The decrease in blood cells showed the damages caused by phenol to these blood cells or interception in the synthesis process and hemolysis of erythrocytic cell (Akmal et al., 2026; Tilak et al., 2007). These observations were supported by Tilak et al. (2007), Muthukumaravel et al. (2023) and Akmal et al. (2024). Decrease level of cholesterol in both exposure due to increase degree of demand for cholesterol through cortico-steroidogenesis raw material of steroid hormone (Zaki et al., 2011). These present findings agree with Zaki et al. (2011) and Shabbir et al. (2024). Level of VLDL cholesterol decrease in liver due to possible damage to liver and less amount of triglyceride supply to body tissue. Level of triglycerides, LDL cholesterol and HDL cholesterol decrease in experiments because of increase demand of energy to overcome stress very low amount of energy store. These results were supported by S. Ahmad et al. (2024a) and Shahzadi et al. (2024). These observations were also supported by phenol effect on rat model with (Kamal-Eldin et al., 2000). Significance elevation noted in AST and ALT as compared to control group in both experiments due to damages of liver these enzymes are not under control. These enzymes are present in the mitochondria and cytosol of hepatocytes (Ohgo et al., 2009) increased level showed the damages due to poisoning (S. Ahmad et al., 2024b), degeneration and necrosis of hepatocytes. These enzymes

involve in metabolism of amino acid to provide energy. Energy demands increase hence their level increase. Same findings observed by Zaki et al. (2011). Significant level of reduction in Total protein and Albumin in both low dose 0.30 mg/L and in high dose 0.40 mg/L. The decreased levels suggested that due to high energy demand, protein may be used as alternative form of energy due to toxicity caused by phenol therefore, level reduced in blood. Our results are same as Monfared and Salati (2013) and Hori et al. (2006). This study revealed significant reduction in globulin level in phenol exposure groups which showed that immunity suppress due to this toxic phenol as this protein play role in immunity. Similar findings were recorded in the previous study by Zaki et al. (2011). Significance high reduction in blood glucose level was recorded after exposed to low and high dose of phenol because of decrease synthesis of carbohydrate and increase energy demand of glucose. Persistent exposure to phenol for 28 days might result in a considerable drop in blood glucose as stored intracellular glycogen in the liver is entirely depleted. This hypoglycemia happens when the fish's metabolic demand, which is commonly caused by high energy requirements or environmental stress, exceeds the rate at which glucose enters the circulation. As a result, the breakdown of carbohydrate metabolism and failure to maintain a dynamic equilibrium cause energy stores to be depleted (Moraes et al., 2016). These present findings also agreed with Remya et al. (2014) and El-Sayed et al. (2007). T3 and T4 increase but TSH significantly decrease in phenol treated group in both low and high dose due to high levels of thyroid hormone are eliminated from the blood as a result of increased biliary excretion of thyroid hormone and deiodination (Akter et al., 2024) and increase demand of ATP for the production of energy in the presence of stress. These observations are supported by Gad and Saad (2008). Singh and Singh (1980) also observed significant reduction in TSH in serum due to thyroid dysfunctions. A functional negative feedback loop inside the HPT axis is shown by the observed rise in T3 and T4 levels and corresponding drop in TSH. higher circulating thyroid hormones limit TSH release to control energy mobilization in this endocrine shift, which is a physiological reaction to the higher metabolic demands brought on by phenol-related acute stress. Consequently, this compensatory response ensures that the fish can sustain the energy costs associated with detoxification and homeostatic maintenance (Peter, 2011; Rathore et al., 2025). Significant alleviation observed in urea level in blood which shows that kidney is not working well. Level of Blood urea nitrogen and Creatinine also high demonstrated that reduction of blood flow or filtration in kidney (S. Ahmad et al., 2024c).

Liver is main organ for the detoxification of toxicants, so it is mainly affected after exposure to phenol both structurally and functionally. Histology of normal liver tissue shows nucleus in the lateral position, regular shaped hepatocytes and normal hepatic vein. Histopathology of phenol treated liver show pyknotic nuclei, cytoplasmic vacuolation, eosinophilic granules in cytoplasm and necrosis which was more severe in high dose same findings were observed by Jamil et al. (2024), Ibrahem (2012) and Muthukumaravel et al. (2021). Kidney is directly involved in the excretion of phenol from blood, maintains normal pH of blood and reabsorption of fluids. Kidney cells undergo damages in order to overcome normal concentration of phenol from blood. Histological studies of kidney showed sinusoidal spaces, elongated tubules, cluster nuclei formation, Melano-macrophages and damaged parenchyma. Same observation were noted by Muthukumaravel et al. (2021), I. Ahmad et al. (2024), and Hasnain Akmal et al. (2025), and Butchiram et al. (2013) in comparison to control group which shows normal organization of parenchyma and glomerulus of kidney tissue. Gill is main organ for the respiration and osmoregulation of fish. Damaged in gills morphology due to phenol exposure directly affect the osmoregulatory and respiratory efficiency of gills result in reduced consumption of oxygen. Imbalance in gas exchange and ions regulation also occur. Histology of normal gill tissues showed proper arrangement of lamellae and epithelial cell but histology of phenol treated group show curved gill lamellae, oedema, lamellar aneurysm, lamellar disorganization, Bone cell deformities and hyperplasia of epithelial cell. Results of this study was same as Remya et al. (2014), Ibrahem (2012), and Chezhian et al. (2010). Muscles were in direct contact with this contaminated water that causes histological changes in the fish muscle. Histology of Control group shows normal blood cells and muscle tissues but treated group shows pigmented cells around blood cells, necrosis, intermyofibrillar, oedema and atrophy in muscle tissue due to toxicity of phenol**.** Similar findings were made by several researchers who have examined the effects of various contaminants on fish muscle (Jaunjal and Gaupale, 2025).

## CONCLUSION

The results of present study concluded that phenols are potentially damaging for fish. It causes alterations in hematology, biochemical parameters like cholesterol, blood glucose and thyroid hormones. They also cause histological changes in liver, kidneys, gills and muscles. Their effect on hepatocytes in liver also confirmed by the increase secretion of two liver enzymes ALT and AST.

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## AUTHORSHIP CONTRIBUTIONS

All authors contributed equally to this work.

## CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

## ETHICAL APPROVAL

Ethical approval was taken from the University of Okara ‘s Ethical Committee for the current research work (Reference no. UO/ETH/2025/misc.).

## DECLARATION OF AI USE

The manuscript was not prepared using generative AI tools. Nonetheless, the writers are fully responsible for the accuracy of any data, concepts, interpretations, and conclusions presented.

## DATA AVAILABILITY

Data will be available upon request.

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