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# EFFECTS OF TEBUCONAZOLE ON LIVER AND INTESTINE OF FRESHWATER FISH *Cyprinus carpio* (Linnaeus, 1758)

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## ABSTRACT

**The wide application of fungicides in agriculture and human medicines have raised environmental concerns and potential impact on aquatic ecosystem. Tebuconazole is a broad-spectrum fungicide used as curative and protective against several fungal pathogens. However, owing to its environmental persistence, it can have long term adverse effects. The present study was aimed to assess the toxic effects of tebuconazole on the digestive system of fish carp *Cyprinus carpio*. The fish were exposed to 6.47 and 8.09 µL $L^{-1}$ concentrations of tebuconazole for 30 days. Biochemical parameters *viz*., lipid peroxidation, protein content, glutathione-s-transferase and phosphatases were determined in the liver and intestine of carp on exposure to tebuconazole. Histopathological analysis was also performed. Reduction in protein and glutathione-s-transferase was observed in time- and dose-dependent manner which may cause rise in lipid peroxides and oxidative stress. Acid and alkaline phosphatase showed elevated activity during the entire exposure time. Significant alterations in the histology of hepatic and intestinal tissues were observed including nuclear alteration, vacuolation and cytoplasmic degeneration in hepatocytes and mucosal alterations in intestine. The result revealed that tebuconazole not only altered the enzymatic activity but also caused oxidative stress and cellular damage. The results confirm the severe damage to fish organs due to tebuconazole, thereby revealing potential impact on aquatic environment.**

**Keywords:** Aquatic ecosystem, *Cyprinus carpio*, histopathology, tebuconazole

## INTRODUCTION

Global issues like changes in climate and agricultural practices have adversely impacted the water quality, which is a critical issue in the twenty-first century. Release of chemicals used in agriculture, such as pesticides, fertilizers, heavy metals and biosolids in the environment pollute the water sources. Pesticides can be harmful due to their persistence, fate, and transport. In addition to the contamination of soil and water and developing pest resistance, the pesticides have potential to harm non-target species including fish and other aquatic life. Fish are sensitive to the changes in water and are widely used as model indicator organisms for aquatic pollution (Das and Chakrabarty 2007). The degree of pesticide toxicity in fish varies widely depending upon the types, forms, species, and other factors. Pesticides directly affect aquatic organisms by causing massive mortality or destroying their food source and indirectly by causing detrimental effects on fish growth and their survival (Helfrich *et al.,* 2009). A large number of freshwater systems are affected by pollutants found in wastewater releases, thus many critical ecological services and biodiversity are lost (Bertahas *et al.,* 2006).

Tebuconazole (TBZ) is a triazole fungicide and widely used in agriculture due to its protective and curative action. It is classified as a possible human carcinogen and is detrimental to aquatic life. It reportedly causes endocrine disruption, electrolyte imbalance, oxidative stress, and developmental

toxicity among other adverse effects in aquatic organisms (Yu *et al.,* 2013; Li *et al.,* 2019, 2020; Subbiah *et al.,* 2020). Toxic substances bioaccumulate and cause redox reactions that produce free radicals, particularly free oxygen radicals and other reactive oxygen species (ROS), which change the biochemistry of fish tissues (Narra, 2016). The antioxidant defence system is one of the most easily activated mechanisms. Oxidative stress, caused by an imbalance between the generation and removal of ROS, can lead to oxidative damage to multiple cellular targets if ROS production exceeds the anti-oxidative action of cells.

Lipid peroxidation is a commonly used indicator of oxidative stress, serving as a marker of ROS-induced damage to the cell membrane. The relationship between the generation of oxidants and their elimination or scavenging by antioxidant mechanisms determines the degree of lipid peroxidation (Jos *et al.,* 2005). Antioxidant responses have been proposed within aquatic organisms as biomarkers of exposure to environmental pollutants (Yang *et al.,* 2020). Glutathione-s-transferase is a member of a multifunctional phase II biotransformation enzyme family that is found in the cytosol of most cells. It catalyses the conjugation of tripeptide glutathione to various compounds with an electrophilic group (George and Buchanan, 1990). Acid phosphatase has been identified as a marker enzyme for the detection of lysosomes in cell fraction; and alkaline phosphatase is a common enzyme bound to the plasma membrane which is used to evaluate the plasma membrane's integrity (Jiang *et al.,* 2012). Any change in these enzymes affects the antioxidant and metabolic activity of fish.

Histopathological alterations have extensively been employed as biomarkers in both laboratory and field studies to assess the health of fish exposed to pollutants. Examining particular target organs is possible by using histopathological biomarkers, which advantageously help in environmental monitoring (Hadi and Alwan, 2012; Latif *et al.,* 2013). Liver is the organ involved in detoxification and breakdown of toxic substances into their metabolites so deactivates them; some of these chemicals end up in tissue. Liver is the primary location for storing high-energy foods (glycogen, lipids) and plays a role in digestion and physiological function. Changes in liver could be useful indicators of prior exposure to environmental stressors. Gastrointestinal tracts of fish are susceptible to toxic pollutants that can damage the gut structure and function when ingested through food and water (Banerjee and Bhattacharya, 1995).

*Cyprinus carpio*, commonly known as common carp, is a freshwater fish species widely used in aquaculture. The perusal of literature has revealed more focused research on short-term or acute exposures to fish and also a significant gap in the studies specifically examining their impact on intestinal tissues, which play a vital role in nutrient absorption and immune response (Clasen *et al.,* 2018). *C. carpio* is used as a preferred model organism in toxicological studies due to its easy availability, high adaptability and stress tolerance. The exposure of this fish to sub-lethal concentrations of fungicides results in significant biochemical and histopathological alterations that cause oxidative stress and impair organ function. Hence, the present study was aimed to assess the impact of tebuconazole exposure on the liver and intestines of fish. The study also evaluated the antioxidant levels and histopathological consequences of tebuconazole exposure on common carp *C. carpio* for 30 days.

## MATERIALS AND METHODS

In this study, *Cyprinus carpio* was selected as the model organism due to its easy availability and resilience to varying environmental conditions. The present study was conducted in 2021-2022 at Animal Physiology Laboratory, Department of Biosciences, Himachal Pradesh University, Shimla (India). The fish *C. carpio* weighing 150-200 g and measuring 20 ± 2 cm in length were purchased from Fish Seed and Breeding Farm Deoli, district Bilaspur, Himachal Pradesh (India), located at a latitude of 31°22'33"N and longitude of 76°48'32"E. The fish were kept in 0.2% potassium permanganate solution for 2-4 min to check for any damage or infection.

***Experimental design***

The experiment was carried out after proper approval from the Himachal Pradesh Fisheries Department. The fish were kept in a glass aquarium with 80 L dechlorinated tap water for 15 days to allow them to adapt to the new environment. They were fed commercial supplemental feed twice a day at 10:00 and 17:00. Excreta and leftover food were siphoned every day. The water exhibited the following physicochemical characteristics: temperature 22 °C, pH 7.8 ± 0.2, dissolved oxygen 8 ± 2 mg $L^{-1}$, and total dissolved solids 150 ± 8 ppm (determined by using water analyser kit, model CK 710). Following acclimatization, all fish were divided into 3 groups. Group I fish were kept in normal water as control. Groups II and III fish were kept in water tanks with 6.47 and 8.09 µL $L^{-1}$ concentrations of tebuconazole (i.e. $1/5^{th}$ and $1/4^{th}$ $LC_{50}$ of 96 h). The $LC_{50}$ value of tebuconazole (25.9% EC) for 30 days was 32.37 µL $L^{-1}$.

***Histopathological examination***

The liver and intestine of control and treated fish were fixed in Bouin's fixative for 24 h. After fixation, the tissues were paraffinized for histological study. Sections of 5-6 µm size were cut on a rotary microtome, stretched on albuminized slides and dewaxed. These sections were stained with hemato-xylin-eosin, mounted in DPX and viewed under a Leica microscope OSM 2 with digital camera.

***Biochemical analysis***

Biochemical analysis of liver and intestine of the fish exposed to toxicant and of control was done on $10^{th}$, $20^{th}$ and $30^{th}$ days. Fish were sacrificed by head injury and the samples collected and kept in ice. Lipid peroxidation, total protein, glutathione-s-transferase, and acid and alkaline phosphatase were analysed. Lipid peroxides were estimated by using thiobarbituric acid as per the method of Dhindsa *et al.* (1981). Protein was estimated by Bradford's method at 595 nm spectrophotometrically (Bradford, 1976). Glutathione-s-transferase (GST) activity was monitored at 340 nm using CDNB as a substrate which reacts with GSH to form a conjugate (Habig *et al.,* 1974). Acid (ACP) and alkaline phosphatase (ALP) activities were assessed as per Weil and Russel (1940) in terms of the amount of inorganic phosphate released $mg^{-1}$ protein.

***Statistical analysis***

The data were expressed as mean ± SEM. One-way analysis of variance, followed by post hoc t-test was used to analyse the results. The data were assumed significant at $^{**}p < 0.01$ and $^{*}p < 0.05$. All the data was analysed using SPSS 16 software.

## RESULTS AND DISCUSSION

Among biochemical parameters, lipid peroxidation is considered as a marker of oxidative stress in organisms. As polyunsaturated fatty acids are more susceptible to free radical attack, measuring the peroxidation products of lipids is a common method of assessing the oxidative damage. The process of lipid peroxidation typically involves a chain reaction resulting in the formation of multiple intermediate compounds such as lipid hydroperoxides and malondialdehyde, which are commonly known as reactive oxygen metabolites, and have widely been used as oxidative damage biomarkers (Mateos and Bravo, 2007). A raised level of thiobarbituric acid reactive substances (TBARS) was noticed in the liver and intestine after exposure to TBZ (Table 1). A significant increase in TBARS was observed at both concentrations with increasing exposure period, suggesting excessive accumulation of reactive oxygen species and inhibition of antioxidative machinery which can lead to oxidative stress (Dinu *et al.,* 2010; Wang *et al.,* 2020). Similar time- and dose-dependent increase was also reported in various fish species due to oxidative stress caused by pesticides (Toni *et al.,* 2011; Kumar *et al.,* 2019).

**Table 1: Lipid peroxidation level (n moles of TBARS formed $g^{-1}$ fresh tissue weight) in liver and intestine of normal and tebuconazole-treated *Cyprinus carpio* from 10-30 day's period. The values are mean ± SEM; n = 6 in each group (**p <0.01; * p <0.05)**

| Groups | Lipid peroxidation level (n moles of TBARS* formed $g^{-1}$ fresh tissue weight) | | | | | |
|---|---|---|---|---|---|---|
| | Liver | | | Intestine | | |
| | 10 days | 20 days | 30 days | 10 days | 20 days | 30 days |
| Control | 3.03 ± 1.30 | 3.20 ± 0.53 | 3.17 ± 1.02 | 2.52 ± 0.98 | 2.54 ± 0.15 | 2.51 ± 0.07 |
| 6.47 µL $L^{-1}$ TBZ | 3.68 ± 0.97* | 3.86 ± 0.84* | 4.93 ± 1.13** | 2.99 ± 0.28* | 3.29 ± 0.11** | 3.74 ± 0.17** |
| 8.09 µL $L^{-1}$ TBZ | 3.83 ± 1.02* | 4.93 ± 0.66** | 6.13 ± 0.94** | 3.23 ± 0.09* | 3.87 ± 0.33** | 4.01 ± 0.13** |

*TBARS = Thiobarbituric acid reactive substances

A decline in the level of protein was observed in the liver and intestine of fish exposed to TBZ as compared to the control (Table 2). Time and dose dependent decline in protein content was seen in the tissues. Protein depletion in tissues during pesticide stress may be a physiological or compensatory mechanism that provides intermediates to the Kreb's cycle. Additionally, it has been proposed that histopathological damage and hydromineral imbalance are the causes of elevated protease activity during pesticide stress. A similar decrease in the protein level was seen in the tissue of fish *Labeo rohito* exposed to malathion (Patil and David, 2013). There was a decrease in the amount of protein in the intestine when *Anabas testudineus* were exposed to monocrotophos. The reduction in protein levels could be caused by the disintegration of proteins into amino acids and the suppression of the machinery involved in protein synthesis (Yadav *et al.,* 2019). While *Danio rario* acutely exposed to TBZ showed no changes in protein level (Sancho *et al.,* 2010), *Cyprinus carpio* was reported to witness a decrease in protein level (Toni *et al.,* 2011). It was observed that the effects of tebuconazole on fish differ depending on the exposure circumstances and are species specific.

**Table 2: Protein content (mg $g^{-1}$ fresh tissue weight) in liver and intestine of normal and tebuconazole-treated *Cyprinus carpio* from 10-30 day's period. The values are mean ± SEM; n = 6 in each group (**p <0.01; * p <0.05)**

| Groups | Protein content (mg $g^{-1}$ fresh tissue weight) | | | | | |
|---|---|---|---|---|---|---|
| | Liver | | | Intestine | | |
| | 10 days | 20 days | 30 days | 10 days | 20 days | 30 days |
| Control | 83.15 ± 0.41 | 84.23 ± 0.83 | 85.17 ± 1.03 | 54.35 ± 0.48 | 55.46 ± 0.83 | 54.17 ± 0.92 |
| 6.47 µL $L^{-1}$ TBZ | 81.28 ± 0.56* | 76.24 ± 0.12** | 70.34 ± 0.93** | 51.71 ± 0.87* | 48.29 ± 1.25** | 46.73 ± 1.13** |
| 8.09 µL $L^{-1}$ TBZ | 80.04 ± 0.43* | 73.17 ± 0.71** | 67.72 ± 0.86** | 51.18 ± 1.15* | 47.01 ± 0.93** | 44.95 ± 1.53** |

Glutathione-s-transferase (GST) enzyme activity is presented in Table 3. GST activity showed increase at a concentration of 6.47 µL $L^{-1}$ TBZ after 10 days in the liver and intestine; however, increasing the exposure period to 30 days caused decline in the enzyme activity. On exposure to a higher dose of 8.09 µL $L^{-1}$ TBZ, an increase in the enzyme activity was seen at 10 day's stage, and a decline in enzyme activity was seen with an increase in exposure period in liver as compared to the control. A significant reduction in GST activity was noticed in intestine after 10 to 30 day's exposure

**Table 3: GST activity (µ mole of GSH conjugate formed $min^{-1}$ $mg^{-1}$ protein) in liver and intestine of normal and tebuconazole-treated *Cyprinus carpio* from 10-30 days period. The values are mean ± SEM; n = 6 in each group (**p <0.01; * p <0.05)**

| Groups | GST (µ mole of GSH conjugate formed $min^{-1}$ $mg^{-1}$ protein) | | | | | |
|---|---|---|---|---|---|---|
| | Liver | | | Intestine | | |
| | 10 days | 20 days | 30 days | 10 days | 20 days | 30 days |
| Control | 4.27 ± 0.73 | 4.51 ± 0.28 | 4.72 ± 1.16 | 5.17 ± 0.56 | 5.13 ± 0.14 | 5.20 ± 0.25 |
| 6.47 µL $L^{-1}$ TBZ | 6.14 ± 0.77** | 3.84 ± 0.19* | 3.30 ± 0.75 | 5.56 ± 0.73* | 4.78 ± 1.03** | 4.12 ± 1.17** |
| 8.09 µL $L^{-1}$ TBZ | 7.20 ± 0.16** | 3.07 ± 0.24* | 2.76 ± 0.22** | 4.73 ± 0.49* | 4.46 ± 0.84** | 3.80 ± 0.53** |

period to 8.09 µL $L^{-1}$ TBZ concentration as compared to the control treatment. Initially, GST activity increased as a defense mechanism against oxidative stress. However, this initial rise was followed by a subsequent decline in enzyme activity, which may be attributed to the excessive generation of reactive species surpassing the enzyme's ability to neutralize them. Inhibition of GST enzyme has also been reported in the fish exposed to propiconazole (Tabassum *et al.,* 2016). Overproduction of free radicals, which may act as their own substrate, can inhibit these antioxidant-protective enzymes. Reductions in protein synthesis or other inhibitions that impact gene expression could also be the cause of the observed decrease in activity (Oruc, 2012). A decrease in GST activity due to pesticide exposure may lead to oxidative imbalance and failure of detoxification machinery (Tabassum *et al.,* 2016; Zahran *et al.,* 2018).

**Table 4: Acid phosphatase activity (µMPi $mg^{-1}$ protein) in liver and intestine of normal and tebuconazole-treated *Cyprinus carpio* from 10-30 days period. The values are mean ± SEM; n = 6 in each group (**p <0.01; * p <0.05)**

| Groups | Acid phosphatase (µMPi $mg^{-1}$ protein) | | | | | |
|---|---|---|---|---|---|---|
| | Liver | | | Intestine | | |
| | 10 days | 20 days | 30 days | 10 days | 20 days | 30 days |
| Control | 18.34 ± 0.58 | 18.36 ± 0.57 | 18.37 ± 0.75 | 8.21 ± 0.45 | 8.18 ± 0.57 | 8.22 ± 0.23 |
| 6.47 µL $L^{-1}$ TBZ | 20.17 ± 0.26$^{*}$ | 22.56 ± 0.72$^{**}$ | 27.02 ± 0.36$^{**}$ | 8.76 ± 1.13 | 9.43 ± 1.03$^{*}$ | 10.66 ± 0.87$^{**}$ |
| 8.09 µL $L^{-1}$ TBZ | 22.13 ± 0.33$^{**}$ | 25.57 ± 0.36$^{**}$ | 30.35 ± 0.68$^{**}$ | 8.95 ± 0.95 | 9.76 ± 0.97$^{**}$ | 11.17 ± 0.76$^{**}$ |

The levels of tissue-damaging enzymes like alkaline phosphatase (ALP) and acid phosphatase (ACP) were significantly elevated in the liver and intestine of TBZ-treated fish as compared to the control fish (Table 4, 5). An increase in the enzyme activity was seen with increasing TBZ concentration and exposure period in tissues. Acid and alkaline phosphatases are lysosomal hydrolytic enzymes. Increased phosphatase levels were indicative of tissue damage brought on by a breakdown in the integrity and permeability of the membrane (Dey *et al.,* 2016). A similar rise in ACP and ALP was reported in *Cyprinus carpio* on exposure to chromium (Shaheen and Akhtar, 2012). *Channa punctatus* may have higher hepatic levels of these enzymes on exposure to mercurial fungicide and led to hepatocyte degeneration and lysosomal rupture which causes the enzymes to accumulate in the liver (Ram and Sathyanesan, 1987).

**Table 5: Alkaline phosphatase activity (µMPi $mg^{-1}$ protein) in liver and intestine of normal and tebuconazole-treated *Cyprinus carpio* from 10-30 days period. The values are mean ± SEM; n = 6 in each group (**p <0.01; * p <0.05)**

| Groups | Alkaline phosphatase (µMPi $mg^{-1}$ protein) | | | | | |
|---|---|---|---|---|---|---|
| | Liver | | | Intestine | | |
| | 10 days | 20 days | 30 days | 10 days | 20 days | 30 days |
| Control | 28.13 ± 0.34 | 28.17 ± 0.17 | 28.17 ± 0.75 | 5.43 ± 0.86 | 5.39 ± 0.66 | 5.42 ± 0.21 |
| 6.47 µL $L^{-1}$ TBZ | 33.16 ± 0.16$^{**}$ | 35.07 ± 0.27$^{**}$ | 38.75 ± 0.15$^{**}$ | 5.72 ± 0.93 | 6.13 ± 0.41$^{**}$ | 7.05 ± 0.34$^{**}$ |
| 8.09 µL $L^{-1}$ TBZ | 36.58 ± 0.27$^{**}$ | 39.24 ±1.03$^{**}$ | 45.75 ± 0.79$^{**}$ | 5.97 ± 0.26$^{*}$ | 6.89 ± 0.64$^{**}$ | 7.96 ± 1.03$^{**}$ |

***Histopathology***

**Liver:** No histopathological changes were observed in the liver of control group. A continuous mass of large hexagonal parenchymal cells, known as 'hepatocytes', was visible (Fig. 1A). Histopathological alterations were noticed in the liver of fish exposed to both concentrations of tebuconazole. Nuclear alterations, vacuolation, and necrotic changes were noticed in the hepatocytes at 10 days stage (Fig. 1B); increased vacuolation, necrosis, karyolysis and clumping of nuclei at some areas were observed after 20 days (Fig. 1C); degeneration of fibrotic tissue and cytoplasm, internal hemorrhage and leukocyte infiltration at 30 days (Fig. 1D) were observed on exposure of fish to 6.47 µL $L^{-1}$ TBZ. The extent of damage to the liver was further increased after the treatment of fish with 8.09 µL $L^{-1}$ TBZ. Nuclear changes such as pyknosis, and clumped and hypertrophied nuclei were seen. Pyknotic and

necrotic changes caused damage in hepatocyte leading to vacuolation and enucleated areas (Fig. 1E). Degenerative changes in cytoplasm, clumping of nuclei and vacuolated region at 20 days (Fig. 1F); severely damaged hepatocytes were observed in the liver of treated carp after 30 days (Fig. 1G). Vacuolation, hypertrophy and vascular dilation were also reported in the Van fish exposed to the same

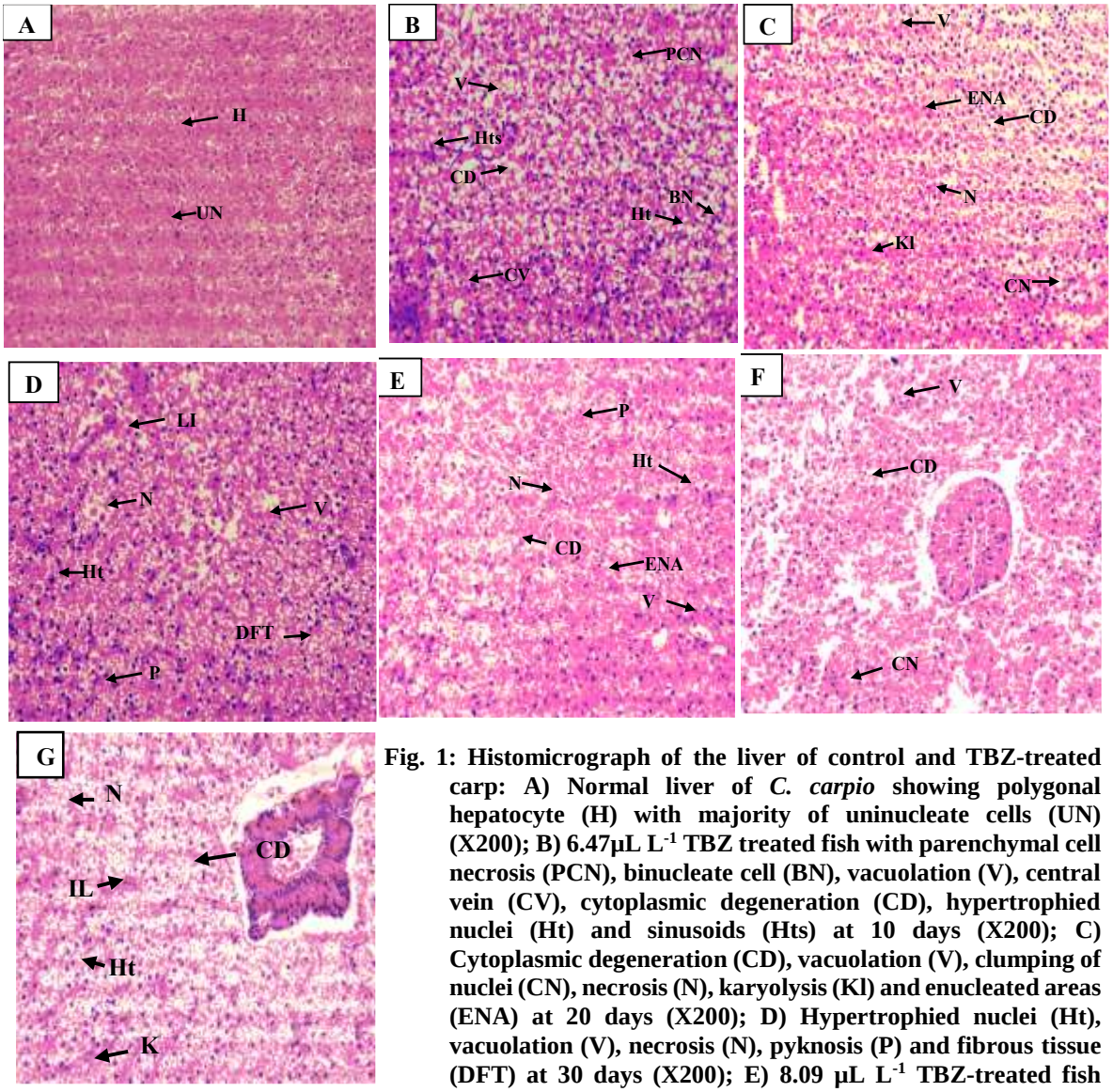


**Fig. 1: Histomicrograph of the liver of control and TBZ-treated carp: A) Normal liver of *C. carpio* showing polygonal hepatocyte (H) with majority of uninucleate cells (UN) (X200); B) 6.47µL $L^{-1}$ TBZ treated fish with parenchymal cell necrosis (PCN), binucleate cell (BN), vacuolation (V), central vein (CV), cytoplasmic degeneration (CD), hypertrophied nuclei (Ht) and sinusoids (Hts) at 10 days (X200); C) Cytoplasmic degeneration (CD), vacuolation (V), clumping of nuclei (CN), necrosis (N), karyolysis (Kl) and enucleated areas (ENA) at 20 days (X200); D) Hypertrophied nuclei (Ht), vacuolation (V), necrosis (N), pyknosis (P) and fibrous tissue (DFT) at 30 days (X200); E) 8.09 µL $L^{-1}$ TBZ-treated fish showing pyknosis (P), hypertrophied nuclei (Ht), necrosis (N), cytoplasmic degeneration (CD), enucleated areas (ENA) and cytoplasmic vacuolation (V) after 10 days (X200); F) Cytoplasmic vacuolation (V), degenerative changes in cytoplasm (CD) and clumping of nuclei (CN) at 20 days (X200); and G) Hypertrophied nuclei (Ht), infiltration of lymphocytes (IL), Kupffer cells (Ku), necrosis (N) and cytoplasmic degeneration (CD) after 30 days (X200)**

fungicide (Oguz *et al.*, 2022). Reduced liver size and degeneration of liver was also found in zebrafish (Li *et al.*, 2019). Our results are in line with a study that reported hypertrophy, necrosis and enucleated hepatocytes in the liver of *Clarias gariepinus* exposed to hexaconazole (Lanjewar *et al.*, 2023). TBZ exposure alters the morphology and function of liver, disturbing the physiology and homeostasis of liver in *D. rario* (Macirella *et al.*, 2022). Cellular enlargement is a common feature of hypertrophy. Increased hepatocyte vacuolation is a sign of a degenerative process that indicates metabolic damage

which may result from exposure to polluted water (Pacheco and Santos, 2002). Degenerative and necrotic alterations generate immune cells and trigger off inflammatory reactions (Troncoso *et al.*, 2012).

**Intestine:** Normal morphology having no pathological changes in the intestine was observed in control fish (Fig. 2A). Infiltration of lymphocytes, vacuolation and muscular destruction were seen in the intestine treated with 6.47 µL $L^{-1}$ TBZ. Also, detachment of the base of villi with loss of their boundaries was noticed at 10 days stage (Fig. 2B). Rupturing and loss of muscularis, vacuolated cytoplasm with increasing mucus secreting cells and degeneration of mucosal and submucosa was

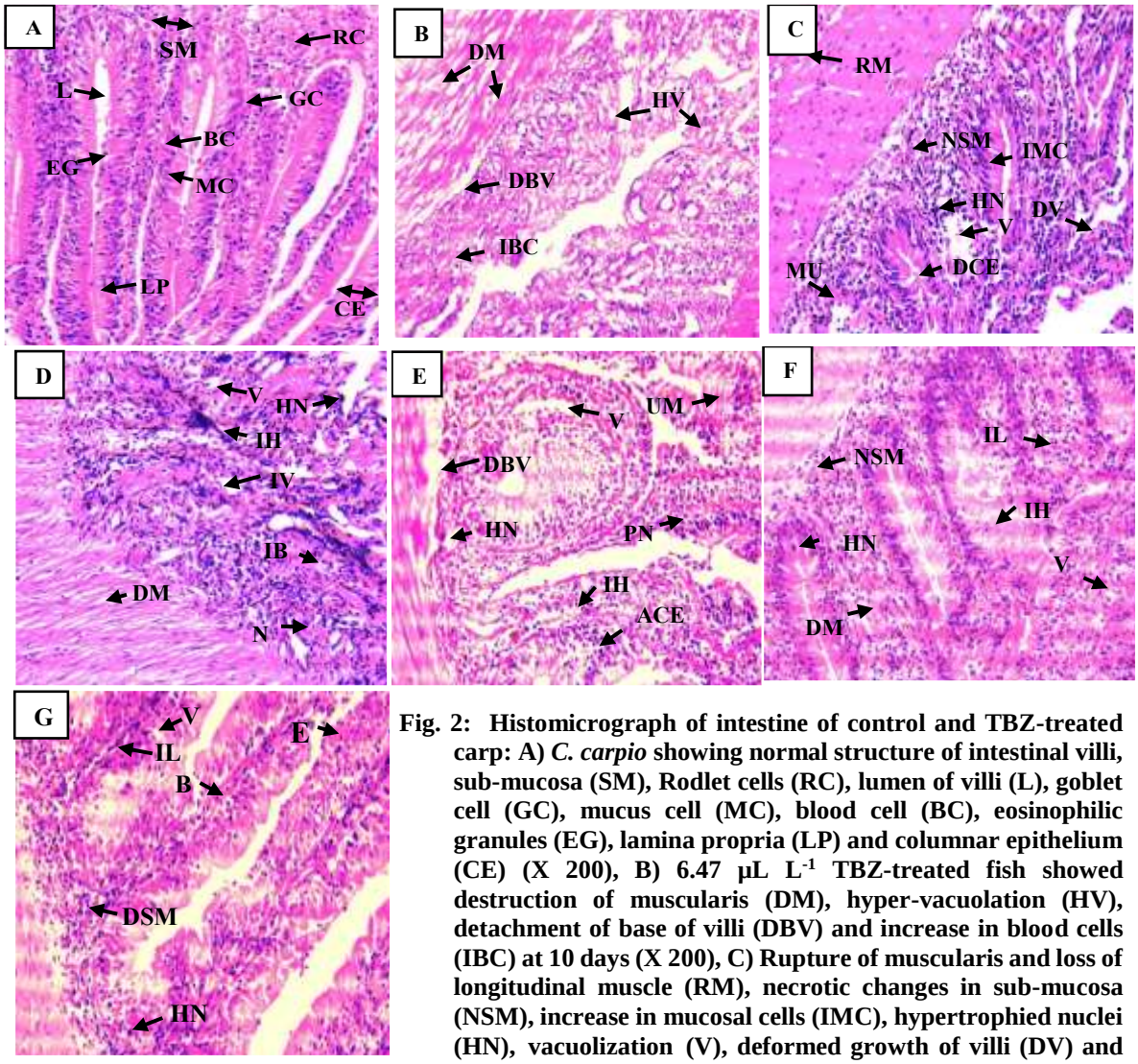


**Fig. 2: Histomicrograph of intestine of control and TBZ-treated carp: A) *C. carpio* showing normal structure of intestinal villi, sub-mucosa (SM), Rodlet cells (RC), lumen of villi (L), goblet cell (GC), mucus cell (MC), blood cell (BC), eosinophilic granules (EG), lamina propria (LP) and columnar epithelium (CE) (X 200), B) 6.47 µL $L^{-1}$ TBZ-treated fish showed destruction of muscularis (DM), hyper-vacuolation (HV), detachment of base of villi (DBV) and increase in blood cells (IBC) at 10 days (X 200), C) Rupture of muscularis and loss of longitudinal muscle (RM), necrotic changes in sub-mucosa (NSM), increase in mucosal cells (IMC), hypertrophied nuclei (HN), vacuolization (V), deformed growth of villi (DV) and degeneration of columnar epithelium (DCE) at 20 days (X 200), D) Damaged muscularis with the loss of circular muscle (DM), necrosis (N), internal hemorrhage (IH), hypertrophied nuclei (HN), hyper-vacuolation (V), increased blood cells (IB) and irregular pattern of villi (IV) at 30 days (X 200), E) 8.09 µL $L^{-1}$ TBZ group revealed vacuolation (V), detachment of base of villi (DBV), ulceration of mucosa (UM), pyknotic nuclei (PN), hypertrophied nuclei (HN), internal hemorrhage (IH) and atrophy of columnar epithelium (ACE) after 10 days (X 200), F) Necrotic changes in sub-mucosa (NSM), increased infiltration of lymphocyte (IL), hyper-trophied nuclei (HN), vacuolization (V), internal hemorrhage (IH) and degenerative changes in the mucosa (DM) after 20 days (X200), and G) increased infiltration of lymphocytes in lamina propria (IL), vacuolization (V), hypertrophied nuclei (HN), edematous columnar epithelium (E), increase in blood cells and internal hemorrhage (B), degenerative changes in sub mucosa (DSM) at 30 days (X200)**

witnessed at 20 days stage (Fig. 2C); increased infiltration of lymphocytes, excessive hemorrhage, irregular pattern and widening of lumen of villi were evident at 30 days stage (Fig. 2D). Mucosal ulceration, hypertrophy, pyknosis and alterations in the columnar epithelium (Fig. 2E), hypertrophy, vacuolation, internal hemorrhage, necrotic and degenerative changes in the mucosal epithelium (Fig. 2F) were observed in the intestine after exposure to 8.09 µL $L^{-1}$ TBZ at 10 and 20 days, respectively. Aggregation of eosinophilic granules, edema, and ulceration of sub mucosa at 30 days TBZ administration (Fig. 2G) causes severe damage to the intestine of carp. Our results are in line with those of Kruatrachue *et al.* (2003) who reported vacuolation, karyolysis and increased number of mucus cells in the intestine secreting excess of mucus in the lumen of the intestine. Mucus may neutralize and detoxify the toxic substances from the body. Infiltration of lymphocytes and atrophic changes in the intestinal epithelium was reported in *Cirrhinus mrigala* exposed to lambda-cyhalothrin (Velmurugan *et al.,* 2007). Similar results were also seen in *Clarias gariepinus* exposed to hexaconazole. Such intestinal alterations probably result in altered fish biological processes, which might cause lethargic behavioral reactions. These impacts also impair the intestine's ability to absorb and digest food (Lanjewar *et al.,* 2023).

Histopathological changes in the digestive system may be correlated with alterations in enzyme activity and increased lipid peroxides levels. Necrosis is frequently linked to the oxidative stress and can also result from enzyme inhibition, cellular membrane integrity damage, protein synthesis disruptions and carbohydrate metabolism abnormalities (Mela *et al.,* 2007). Elevation in LPO levels due to pesticide exposure is directly linked to excessive production of free radicals and damage to lipids, protein, DNA and other molecules. ROS cause increased damage to membranes, which makes them leaky and eventually results in necrosis, cell death, or apoptosis (Lushchak, 2011). An increase in ACP activity may be associated with degeneration of hepatocytes and over-production of leucocytes. Infiltration and tumour formation in intestine and other tissues was also reported (Sastry and Gupta, 1978). ALP is involved in the formation of bones and membrane transport in addition to catalysing the dephosphorylation of phosphorylated organic compounds. Increase in ALP activity was linked to the cell damage and alteration in tissues (Karan *et al.,* 1998; Atli and Canli, 2007). The present study reveals that tebuconazole is highly toxic to the liver and intestine of fish. Further, biomarkers like histopathology, lipid peroxidation levels, protein content antioxidant and phosphatase enzyme activity are effective indicators for chemical biomonitoring and risk assessment in aquatic organisms.

**Conclusion:** Based on the findings of present study, it can be concluded that tebuconazole can induce hepatic and gut histopathological and biochemical changes at even low concentrations, which may result in severe physiological dysfunction and damage in the fish. The presence of tebuconazole in aquatic environment may be harmful to the carp and other aquatic organisms. In addition to a negative impact on fish, pesticide toxicity poses a serious health risk to humans since it bioaccumulates in fish tissues. A regular monitoring program is required to maintain the health of the aquatic environment and to reduce the hazardous effects on the non-target organisms.

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