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Research Article

Toxicological evaluation of histoarchitectural alterations in various tissues of zebrafish (*Danio rerio*) exposed to three sub-lethal concentrations of atrazine

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Abstract
Environmental pollutants, primarily widely used herbicides such as atrazine, pose substantial risks to aquatic ecosystems. Atrazine is commonly detected in surface waters and has been shown to impair growth, physiological processes, and reproductive functions in aquatic organisms. The present study aimed to examine the sub-lethal toxicological effects of atrazine on *Danio rerio* (zebrafish), with particular attention to growth performance, enzymatic biomarkers, and organ-specific tissue damage. Juvenile zebrafish were exposed to atrazine at concentrations of 0.83, 1.25, and 2.5 ppm for 100 days. Growth parameters were measured, and biochemical assays were conducted to assess hepatic and cellular damage marker glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activity. Histopathological analyses of liver, gill, and gonadal tissues were performed to identify structural and functional impairments. Atrazine exposure resulted in a concentration-dependent reduction in body weight and total length, indicating impaired somatic development. Significant increases in GOT and GPT levels were detected, which suggest hepatocellular damage and increased tissue stress. Histological analyses identified severe pathological changes, including hepatocyte degeneration, gill lamellar fusion, and testicular atrophy. Distinct male- and female-specific responses further supported the dose-dependent nature of atrazine-induced toxicity. The findings demonstrate that chronic exposure to sub-lethal concentrations of atrazine induces significant physiological, biochemical, and histopathological disturbances in *Danio rerio*. These results highlight the detrimental impacts of atrazine on fish health and development and emphasize the need for strengthened regulatory controls and continuous monitoring of herbicide contamination in aquatic environments.

Keywords: Atrazine, *Danio rerio*, Enzyme activity, Growth inhibition, Histopathology

INTRODUCTION

Environmental pollution is the contamination of natural ecosystems by harmful physical, chemical, or biological substances that adversely affect living organisms and ecological stability. The primary forms of pollution, including air, water, soil, noise, light, and radioactive pollution, are primarily attributable to industrialization, agricultural practices, and rapid urbanization (Saxena, 2025). Persistent pollutants, such as toxic chemicals,

plastics, and hazardous gases, disrupt ecosystem functions, threaten biodiversity, and pose significant health risks to humans and wildlife. Pesticides such as atrazine can contaminate freshwater systems, causing adverse effects on aquatic organisms, disrupting food webs, and threatening ecosystem stability. Effective pollution management requires implementing sustainable agricultural practices, enforcing stringent environmental regulations, and enhancing public awareness to protect both ecological and human health (Onwudiegwu *et al.,* 2025). Atrazine, a widely used herbicide, inhibits photosynthesis in weeds and is a white, crystalline compound with moderate water solubility and a slow rate of environmental degradation. Its high mobility frequently leads to contamination of soil and surface waters. Atrazine has been demonstrated to cause toxic effects in aquatic species and may function as an endocrine disruptor in both animals and humans (Chang *et al.,* 2022).

Zebrafish (*Danio rerio*) are small freshwater teleosts widely recognised as a valuable vertebrate model organism because of their genetic, physiological, and developmental similarities to humans, including nearly 70% orthology with the human genome. Their rapid development, external fertilization, and transparent embryos facilitate real-time visualization of organogenesis and cellular processes (Akyürek *et al.,* 2025). Zebrafish are cost-effective, straightforward to maintain, and possess conserved biological pathways relevant to human physiology. In toxicology, zebrafish serve as an essential model for evaluating chemical toxicity, environmental hazards, endocrine disruption, and organ-specific damage. Their well-characterised genome, regenerative abilities, and resemblance to human organ systems make zebrafish a robust model for investigating disease mechanisms, drug screening, and the effects of environmental toxicants (Adhish *et al.,* 2023; Destro *et al.,* 2021).

The present study aimed to investigate the toxicological effects of sub-lethal atrazine exposure in *Danio rerio*, with particular focus on growth performance and tissue damage in vital organs, specifically the liver and gills. The study evaluated the impact of atrazine on growth parameters and to assess histopathological alterations, including changes in cellular integrity and tissue architecture in liver and gill tissues, to understand atrazine-induced organ toxicity and its potential environmental risks.

MATERIALS AND METHODS

Experimental fish

Danio rerio (zebrafish) were selected for this study. Mature male and female specimens were obtained from a local ornamental fish supplier (Big Fish Aquaculture, Coimbatore–641005, Tamil Nadu, India) and housed separately in laboratory aquaria before breeding. Despite being commercially sourced, the fish displayed the characteristic phenotype of the wild-type AB strain, which is commonly utilized in toxicological and environmental research due to its stable genetic background and consistent physiological responses. Specimens were acclimated under standardised husbandry conditions, including a controlled water temperature of 27 ± 0.5 °C, a 12:12 light: dark photoperiod, and twice-daily feeding with earthworm pieces. These measures were implemented to ensure optimal health and reproductive readiness.

Experimental procedure

The physicochemical characteristics of the experimental water were maintained within optimal limits throughout the study. Recorded parameters included temperature (27 ± 0.5 °C), pH (6.3 ± 0.4), dissolved oxygen (6.5 ± 0.4 mg/L), salinity (0.6 ± 0.03 ppt), nitrite (0.04 ± 0.008 mg/L), and hardness as $CaCO_3$ (19.2 ± 0.05 mg/L). All measurements were conducted in accordance with the standard procedures outlined in Standard Methods for the Examination of Water and Wastewater (Smol *et al.,* 2025). Newly hatched juveniles were separated from their mothers and transferred to a 100-L glass aquarium. Two hundred hatchlings (0-day-old fry) were randomly assigned to four experimental groups, each containing 20 individuals. Three groups, each with 20 fish, were exposed to sub-lethal concentrations of atrazine: 2.5 ppm ($1/10^{th}$ of the LC_{50}), 1.25 ppm ($1/20^{th}$ of the LC_{50}), and 0.83 ppm ($1/30^{th}$ of the LC_{50}). The remaining group served as an untreated control. All juveniles originated from the same spawning event to eliminate age-related variation. Fish received a commercial granular diet twice daily, and growth parameters (length and weight) were measured at 20-day intervals. The experiment used a static renewal system, with water and atrazine solutions replaced weekly to maintain exposure concentrations. For histopathological and biochemical enzyme analyses, six fish per group were sacrificed at predetermined time points: Day 30, Day 60, and Day 100. Sampling was conducted using simple random selection to ensure representativeness and minimize selection bias. Selected fish were euthanized humanely, dissected, and processed immediately for tissue and enzymatic assessments. The entire experimental design, including exposure and sampling, was replicated three times independently to ensure reproducibility and reduce experimental error (Porteus *et al.,* 2024).

Growth rate study

Zebrafish embryos were obtained in an undifferentiated state and reared under controlled laboratory conditions until 20 days post-fertilization (dpf). At this developmental stage, zebrafish are sexually immature and lack ex-

ternal sexual dimorphism; thus, no sex identification or pre-selection was conducted. Juveniles from a single cohort were randomly assigned to either the control or the atrazine-exposed group to maintain uniform developmental status at treatment onset. Since sex differentiation generally begins after 25 to 30 dpf, all individuals at the start of exposure were considered phenotypically unsexed. Final sex identification was performed at the adult stage (≥ 60 dpf) using established morphological markers, including body shape, colouration, and anal fin characteristics. This experimental design allows any observed sex-ratio (1:1) deviations to be attributed to atrazine exposure during the critical period of gonadal differentiation, rather than to pre-existing group differences. Growth was assessed by measuring body weight and length, following the methodology described by Malik *et al.* (2024). Fish weight was determined using an electronic balance, and body length was measured with a Vernier caliper for the same individuals. In each experimental group, 10 fish were sampled, and the mean values were calculated (Chevalier *et al.,* 2024).

Tissue-damaging enzyme activity

Enzyme activity assays served as early biochemical indicators of hepatocellular injury because they respond rapidly to toxicant-induced metabolic and membrane disturbances (Qadir *et al,* 2024). Liver tissues from both control and atrazine-exposed zebrafish were dissected, rinsed in ice-cold physiological saline, and homogenized (10% w/v) in chilled phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000 × g for 15 minutes at 4 °C, and the resulting supernatants were utilized for all biochemical analyses. Total protein concentration was determined using the Lowry method to normalize enzyme activities (U/mg protein) (Arambašić *et al*., 2013). A panel of diagnostic enzymes commonly used in fish toxicology, including glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), was analyzed to assess hepatocellular integrity and metabolic disruption according to established protocols (Suárez *et al.,* 2015). GOT and GPT activities were quantified using specific transaminase assay kits according to the DNPH-based endpoint protocol. These enzymes are recognized as sensitive biomarkers of hepatocellular integrity because increased activity reflects the release of cytosolic transaminases into surrounding tissues after liver injury (Rangasamy *et al.,* 2020). These assays collectively provided a sensitive biochemical profile of atrazine-induced hepatic stress in zebrafish.

Histopathological analysis

Following 100 days of exposure, zebrafish from all treatment and control groups were euthanized (Abu-Zahra *et al.,* 2025). The gills, liver, and gonads were dissected and fixed in 10% formalin for histological analysis. Gonadal maturity was classified through macroscopic observation. Mature females were identified by the presence of oocytes with light yellow to reddish coloration, indicative of increased ovarian vascularization. Mature males were recognized by enlarged, white testes occupying the body cavity. These assessments facilitated evaluation of potential atrazine-induced effects on reproductive organ development and overall physiological condition (Patel *et al.,* 2024).

Animal ethics approval

All zebrafish (*Danio rerio*) experimental procedures adhered to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The Institutional Animal Ethics Committee (IAEC) reviewed and approved the experimental protocol. The study was conducted under CPCSEA approval number 932/Po/Re/S/06/CPCSEA. A total of 80 adult zebrafish (*Danio rerio*), including 40 males and 40 females, were utilized. Measures were implemented to minimize animal suffering and to use the fewest animals necessary to obtain reliable scientific data.

Statistical analysis

All statistical analyses were performed using SPSS Statistics for Windows, version 16.0 (IBM Corp., Armonk, NY, USA). Data are reported as mean ± standard error (SE). Differences among experimental groups were assessed using one-way analysis of variance (ANOVA). If ANOVA revealed significant differences, pairwise comparisons were performed with the least significant difference (LSD) post hoc test. Statistical significance was defined as $p < 0.05$ compared to the control group.

RESULTS

Growth rate of *Danio rerio* exposed to atrazine

Figures 1 and 2 present the total length and weight of *Danio rerio* reared under control conditions and exposed to atrazine at 0.83, 1.25, and 2.5 ppm for 20, 40, 60, 80, and 100 days, respectively. During the early undifferentiated stages (20–40 days), all groups exhibited a gradual increase in growth. Control fish demonstrated slightly greater lengths (approximately 2.4–3.0 cm) and weights (approximately 0.25–0.35 g) compared to the atrazine-treated groups. By 60 days, when sex differentiation became apparent, control males and females reached approximately 4.2 cm and 1.2–1.3 g, respectively, while the 2.5 ppm group showed the lowest growth (3.5 cm, 0.5–0.7 g). At 80 days, the control group maintained superior growth (length of 4.3 cm, weight of 2.0 g), whereas atrazine exposure resulted in a concentration-dependent reduction, most pro-

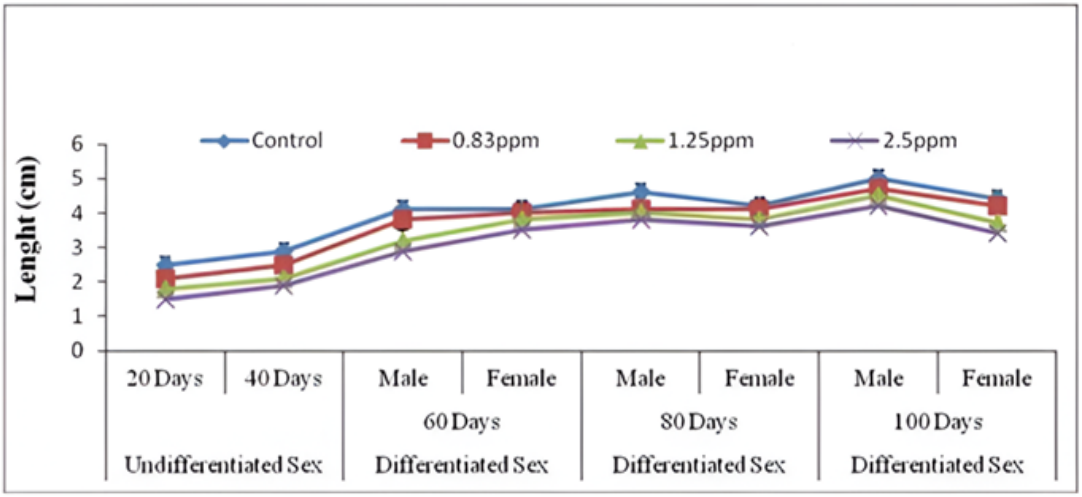

Fig. 1. *Total length of Danio rerio at different concentrations of atrazine*

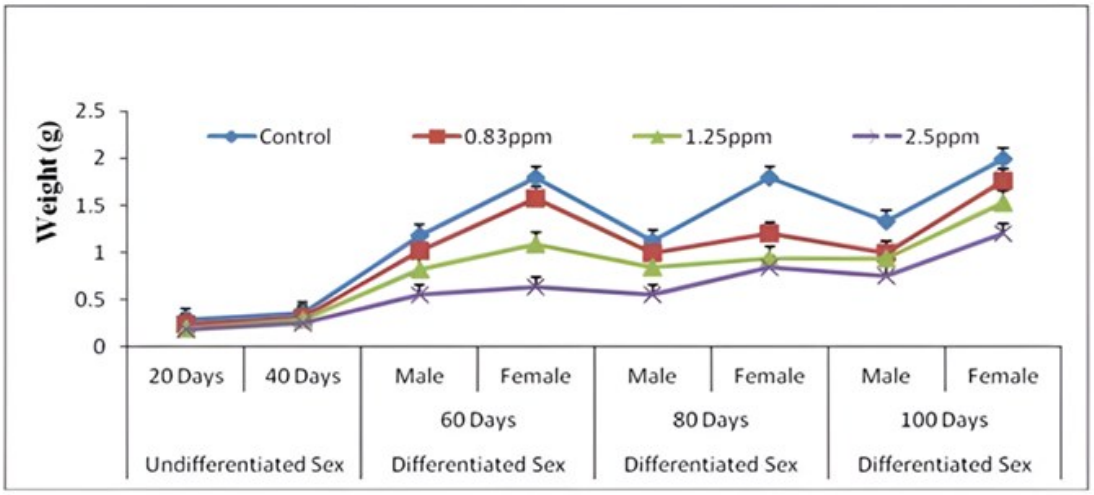

Fig. 2. *Total weight of Danio rerio at different concentrations of atrazine*

nounced at 2.5 ppm. By 100 days, control males and females achieved the highest final length (4.5 cm) and weight (2.0 g). In contrast, the 0.83 and 1.25 ppm groups exhibited moderately reduced growth (4.0–4.2 cm; 1.4–1.7 g), while the 2.5 ppm group recorded the lowest values (3.6–3.8 cm; 1.0–1.2 g), indicating an apparent inhibitory effect of atrazine on long-term growth performance.

Tissue-damaging enzyme activity of glutamate pyruvate transaminase (GPT)

GPT activity increased in a consistent, dose- and time-dependent manner across all examined tissues of *Danio rerio* following atrazine exposure (Figs. 3–5). In the liver, control males and females maintained moderate GPT levels at 100 days (22–24 µmol/mg protein/min). In contrast, exposure to 0.83 ppm and 1.25 ppm resulted in marked increases (38–44 µmol/mg protein/min), with the highest activity observed at 2.5 ppm (approximately 48–52 µmol/mg protein/min in males and 45–50 µmol/mg protein/min in females), indicating pronounced hepatocellular damage. Gonadal tissues also demonstrated substantial elevation in GPT activity with increasing exposure concentration and duration. By the 100th day, control testes and ovaries recorded approximately 17–18 µmol/mg protein/min, whereas the 2.5 ppm group exhibited the highest values (32–36 µmol/mg protein/min in testes and 30–34 µmol/mg protein/min in ovaries). Intermediate increases were noted at 0.83 ppm and 1.25 ppm. Gill tissues showed progressive enhancement of GPT activity. On the 100th day, control males and females exhibited approximately 14–15 µmol/mg protein/min, while the 2.5 ppm treatment resulted in significantly elevated activities (26–29 µmol/mg protein/min). Collectively, the pronounced increase in GPT activity across liver, gonads, and gills indicates severe toxicity resulting from prolonged atrazine exposure, with the most substantial alterations observed at 2.5 ppm.

Tissue-damaging enzyme activity of glutamate oxaloacetate transaminase (GOT)

GOT activity exhibited a distinct concentration- and time-dependent increase in all *Danio rerio* tissues following

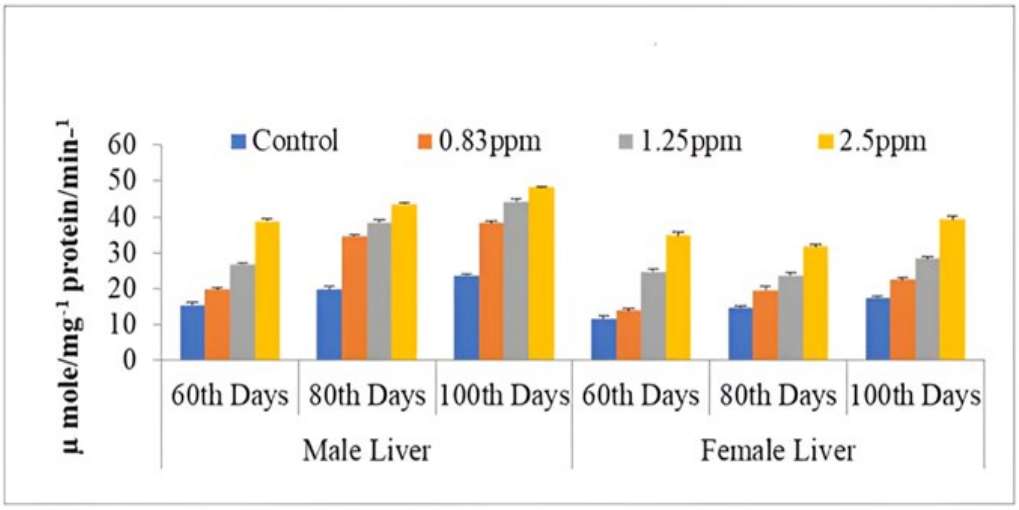

Fig. 3. *GPT activity in the liver of Danio rerio exposed to atrazine*

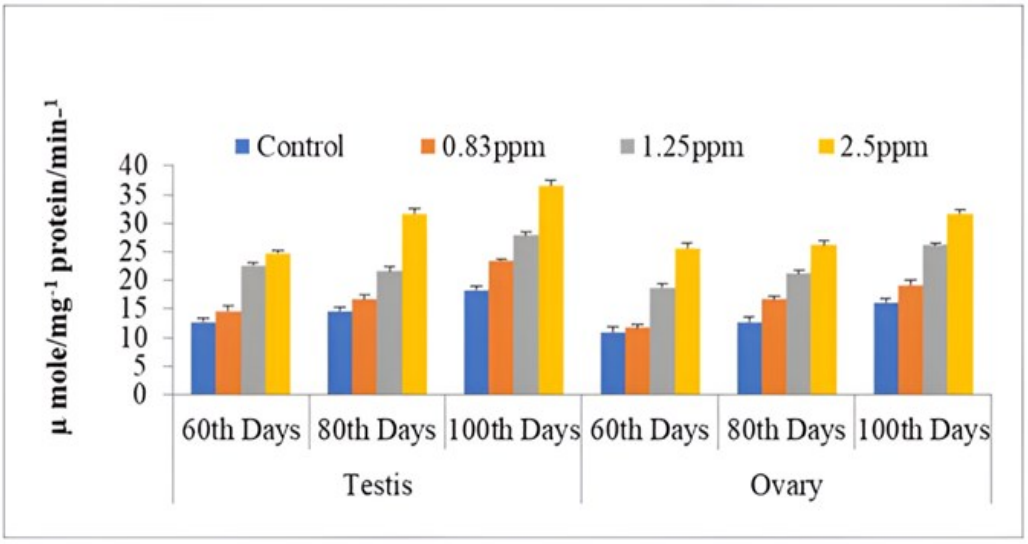

Fig. 4. *GPT activity in the gonads of Danio rerio exposed to atrazine*

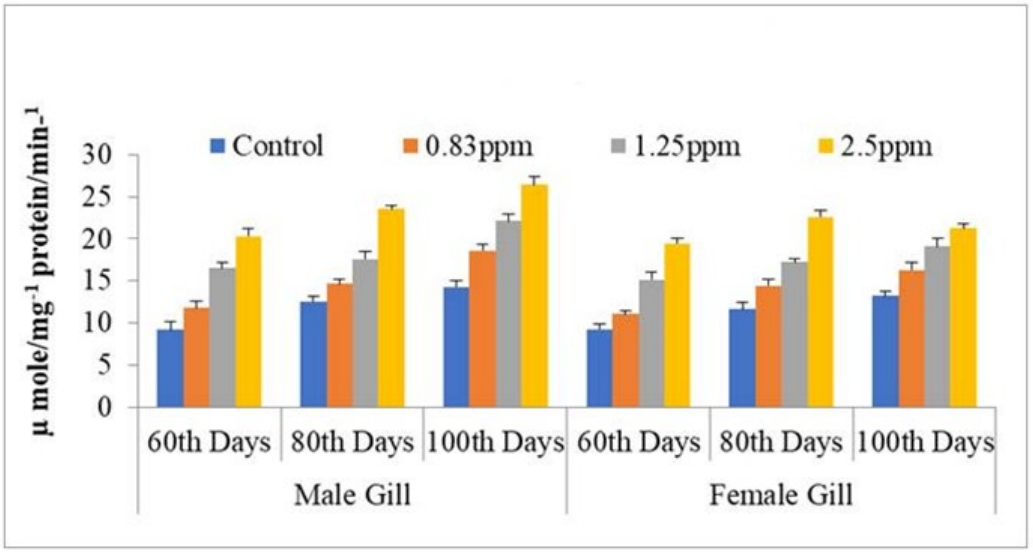

Fig. 5. *GPT activity in the gill of Danio rerio exposed to atrazine*

atrazine exposure. In the liver (Fig. 6), control values on day 100 were approximately 30 µmol mg^{-1} protein min^{-1} in males and 28 µmol mg^{-1} protein min^{-1} in females. Exposure to 0.83, 1.25, and 2.5 ppm atrazine elevated male hepatic GOT activity to approximately 34, 38, and 45 µmol mg^{-1} protein min^{-1}, respectively, while female liver values increased to 33, 36, and 43 µmol mg^{-1} protein min^{-1}. In the gonads (Fig. 7), 100-day control GOT activity was about 25 µmol mg^{-1} protein min^{-1} in testes and 20 µmol mg^{-1} protein min^{-1} in ovaries. Atrazine exposure increased testicular activity to 30, 35, and 40 µmol mg^{-1} protein min^{-1} at 0.83, 1.25, and 2.5 ppm, respectively, and ovarian activity to 28, 31, and 36 µmol mg^{-1} protein min^{-1}, respectively. Gill tissues (Fig. 8) demonstrated similar patterns. On day 100, control levels were 22 µmol mg^{-1} protein min^{-1} in males and 20 µmol mg^{-1} protein min^{-1} in females. Exposed fish showed increased activities of 25, 29, and 35 µmol mg^{-1} protein min^{-1} in males, and 24, 28, and 33 µmol mg^{-1} protein min^{-1} in females at 0.83, 1.25, and 2.5 ppm, respectively. Collectively, atrazine exposure consistently increased GOT activity across all tissues and time points, suggesting progressive tissue damage and enhanced transamination responses with higher concentrations and prolonged exposure.

Histopathological study

Histological analysis showed progressive, dose-dependent liver alterations in both male and female *Danio rerio* exposed to atrazine (Fig. 9 & 10). After 100 days, control groups displayed intact hepatic cords, hepatocytes (H) with clear nuclei, and normal central veins (V). In contrast, atrazine-treated fish exhibited significant pathological changes. Males exposed to higher concentrations exhibited sinusoidal dilation, necrotic degeneration (Nd), disrupted hepatocytes, and acinar cell degeneration (DAc). Females showed similar effects, including vacuolation, blood congestion (BC), widened blood vessels (WBV), necrosis, and distorted hepatic architecture. The most severe changes occurred at 2.5 ppm, with diffuse hepatic degeneration and extensive vacuolization. Atrazine exposure resulted in substantial structural liver damage that increased with concentration and duration, indicating impaired liver function in both sexes.

Progressive, dose-dependent gonadal abnormalities were observed in *Danio rerio* following 100 days of atrazine exposure (Fig. 11 & 12). In control testes, normal spermatogenic stages, including spermatogonia (St), spermatocytes, and compact spermatozoa (Sp), were clearly evident. Treated groups showed increasing disorganisation at higher atrazine concentrations. At 0.83 ppm, mild necrotic zones (Nz) developed, while 1.25 ppm resulted in disrupted seminiferous tubules and reduced sperm density. At 2.5 ppm, severe degeneration was observed, characterized by distorted spermatogenic layers (DST) and degenerated spermatozoa (DSp). Control ovaries contained intact oocytes with normal nuclei (Nu), perinucleolar follicles (PF), and well-organized envelopes (EN). Atrazine exposure led to structural deterioration in the ovaries: at 0.83 ppm, primary oocytes (POF) exhibited cytoplasmic shrinkage; at 1.25 ppm, follicular cell rupture (RO) and lipid infiltration occurred. At 2.5 ppm, extensive oocyte vacuolization (VO) and degenerated ovarian follicles (EOF) indicated pronounced toxic effects.

Histological analysis of *Danio rerio* gills after 100 days revealed distinct structural differences between control and atrazine-treated groups. Gills from control males and females exhibited intact primary lamellae (PL), closely arranged secondary lamellae (SL), and normal chloride cells (CC). In contrast, fish exposed to atrazine developed progressive lesions. At 0.83 ppm, secondary lamellae disorganization and mild hyperplasia (H) were observed. At 1.25 ppm, males exhibited lamellar fusion, curling, and severe vascular congestion (CVS), while females displayed epithelial lifting and disrupted lamellar architecture. Exposure to 2.5 ppm resulted in pronounced damage, including distorted secondary lamellae (DSL), necrotic structural cells (NSC), fusion (F), and aneurysm-like dilations (A). These findings demonstrate that atrazine exposure leads to significant, dose-dependent gill impairment, reducing respiratory efficiency and overall health.

DISCUSSION

Environmental contaminants disrupt essential physiological processes that regulate growth, development, and behaviour in aquatic organisms. Growth indices, including body length and weight, serve as critical biological indicators of health, nutritional status, and the overall condition of individual fish and their populations (Schweizer *et al.,* 2022). The condition factor is frequently used to assess fish health, productivity, and physiological status because it integrates body morphology and growth dynamics (Flura *et al.,* 2022). In this study, a reduction in body weight was observed in *Danio rerio* exposed to atrazine, consistent with previous reports of growth suppression following pesticide exposure. Atrazine-treated fish exhibited slower growth than the control group, indicating significant adverse physiological effects (Figs. 1 and 2) (Tao *et al.,* 2023).

The activities of GOT and GPT were significantly elevated in atrazine-exposed *Danio rerio* compared to the control group. These increases in aminotransferase activity indicate cellular leakage due to chemical-induced tissue injury. The pronounced elevation in GOT and GPT levels suggests that atrazine exposure causes tissue damage, potentially mediated by oxida-

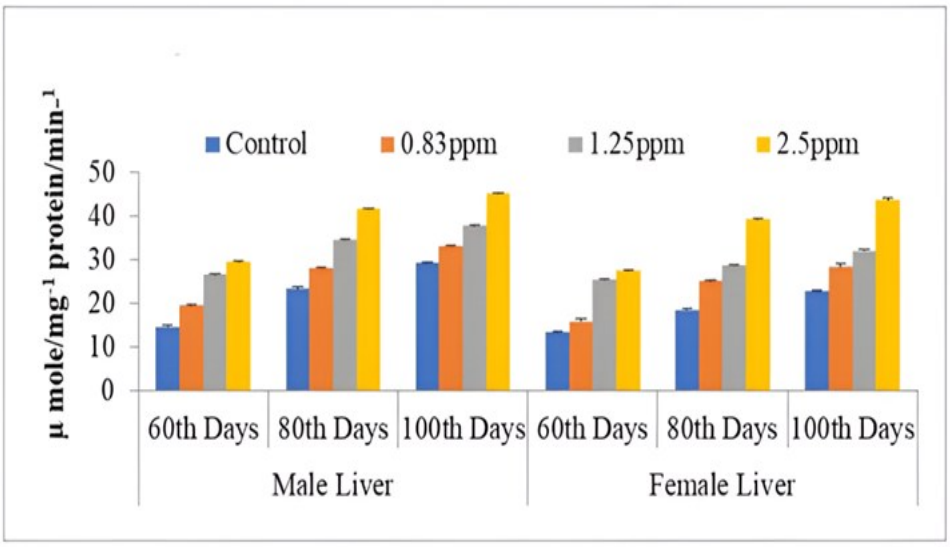

Fig. 6. *GOT activity in the liver of Danio rerio exposed to atrazine*

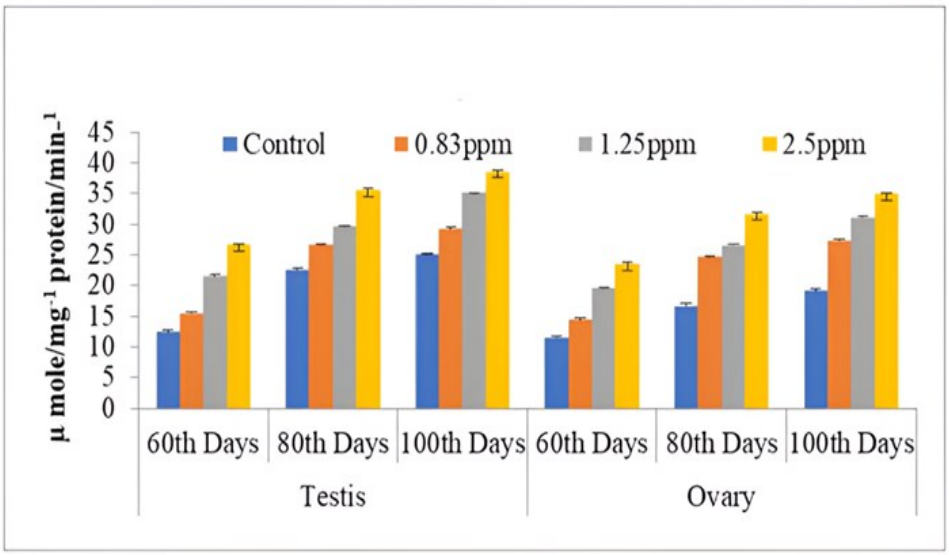

Fig. 7. *GOT activity in the gonads of Danio rerio exposed to atrazine*

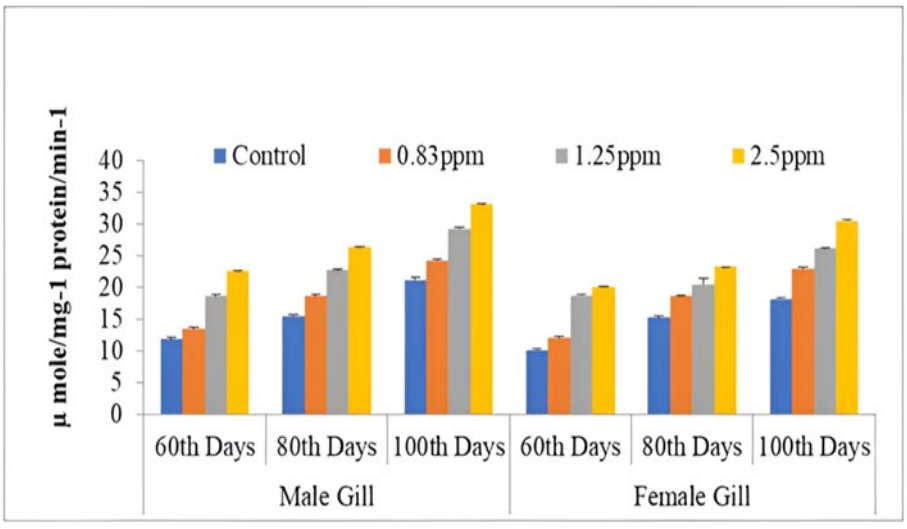

Fig. 8. *GOT activity in the gill of Danio rerio exposed to atrazine*

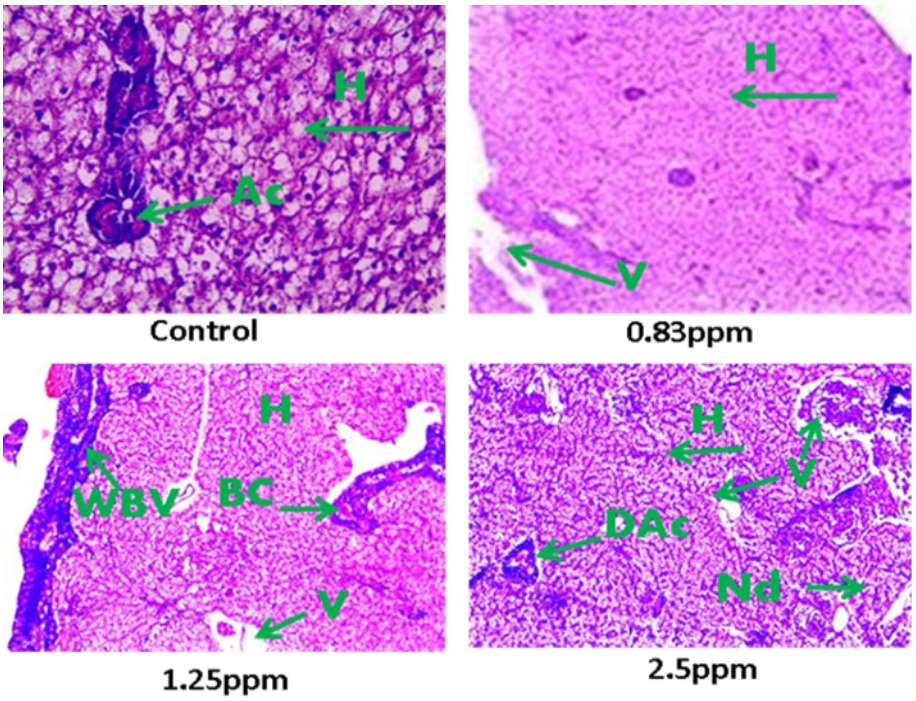

Fig. 9. *Impact of atrazine on the liver of male Danio rerio. Control liver sections showed normal hepatic architecture with well-organized hepatocytes (H) and normal acinar cell arrangement (Ac). Atrazine-treated groups showed progressive histopathological alterations, including hepatocyte disorganization (H), venous dilation (V), widened blood vessels (WBV), bile canaliculus (BC) alterations, degenerated acinar cells (DAc), and nuclear degeneration/necrotic debris (Nd), as indicated by green arrowheads*

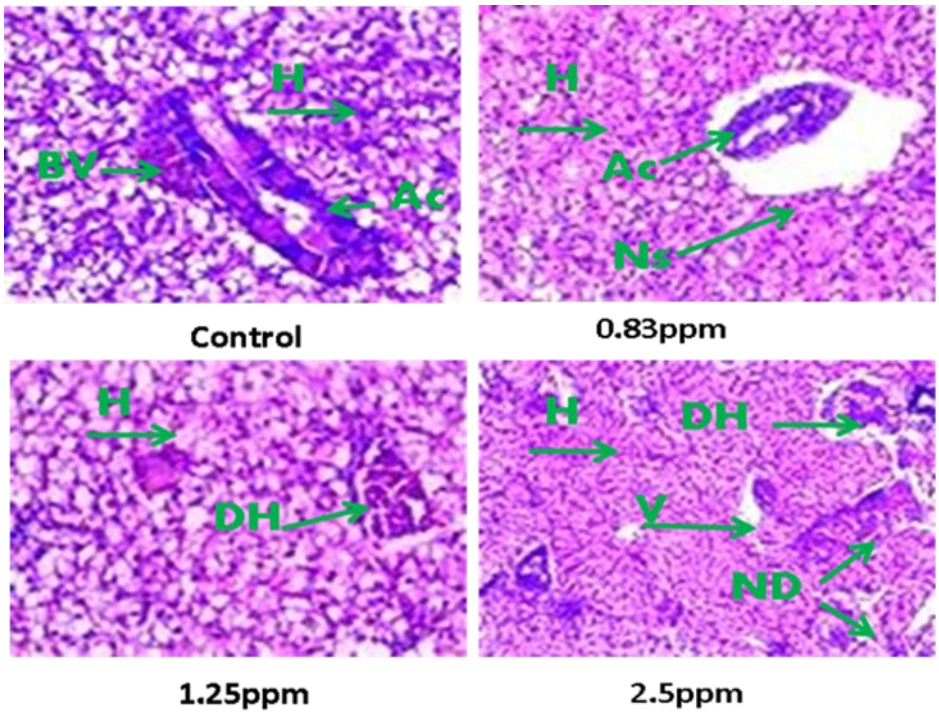

Fig. 10. *Impact of atrazine on the liver of female Danio rerio. Control liver sections showed normal hepatic architecture with well-organized hepatocytes (H), normal acinar cell arrangement (Ac), and normal blood vessels (BV). Atrazine-treated groups showed progressive histopathological alterations, including hepatocyte disorganization (H), nuclear shrinkage (Ns), degenerated hepatocytes (DH), venous dilation (V), and nuclear degeneration/necrotic debris (ND), as indicated by green arrowheads*

tive or free radical mechanisms (Figs. 3-5) (Fan *et al.,* 2025). Moderate cytotoxic effects were detected in the liver, gonads, and gills, and these pathological changes corresponded with the observed biochemical alterations. Comparable findings have been documented in cypermethrin-exposed rats, further supporting the link between pesticide exposure and increased transaminase activity (Shuklan *et al.,* 2023).

Zebrafish juveniles at 20 days post-fertilization (dpf) are in a prepubertal stage, characterized by undifferentiated gonads that are highly responsive to endocrine signals. Atrazine exposure during this critical developmental window appears to increase susceptibility to endocrine disruption, resulting in a female-biased sex ratio and gonadal abnormalities (Ruan *et al.,* 2024). Atrazine has been shown to interfere with steroidogenic pathways and hormone-regulated gene networks, rendering early developmental stages particularly vulnerable. The observed alterations in this study reflect disruption of normal sexual differentiation rather than pre-existing group differences (Abellán-Álvaro *et al.,* 2025).

Enzyme activities increased progressively with higher atrazine concentrations in all examined organs, indicating a clear dose-dependent response. This observation is consistent with Anih *et al.* (2024), who found that variations in metabolic enzymes in fish are directly proportional to lindane exposure levels. GOT and GPT serve as established biomarkers for evaluating pollutant-induced tissue damage in the liver, muscle, and gills (Lozano *et al.,* 2024). Elevated activities of these enzymes in extracellular fluid or plasma are recognized as sensitive indicators of even mild cellular injury, and the increased blood concentrations of GOT and GPT typically indicate hepatocellular damage (Wu *et al.,* 2024). High serum levels of these transaminases are frequently associated with liver dysfunction, inflammation, or necrosis in exposed animals. The observed elevations in enzyme levels in the present study confirm significant atrazine-induced tissue impairment in *Danio rerio* (Figs. 6-8).

The observed elevation of GOT and GPT activities in all examined tissues of *Danio rerio* suggests leakage of these cytosolic enzymes from hepatocytes into surrounding tissues, indicating atrazine-induced hepatic injury. The progressive increase in transaminase activities with higher atrazine concentrations further demonstrates a dose-dependent hepatotoxic effect. These results align with previous findings in *Oreochromis niloticus*, in which exposure to contaminants led to significant increases in GOT and GPT activities (Rangasamy *et al.,* 2020). Elevated transaminase levels are also recognized as early indicators of immune and stress responses during the initial stages of toxicant-induced physiological disruption (Zaman *et al.,* 2025). On the other hand, Yıldırım *et al*. (2025) found an increase in ALT activity in carp (*Cyprinus carpio L.*) after exposure

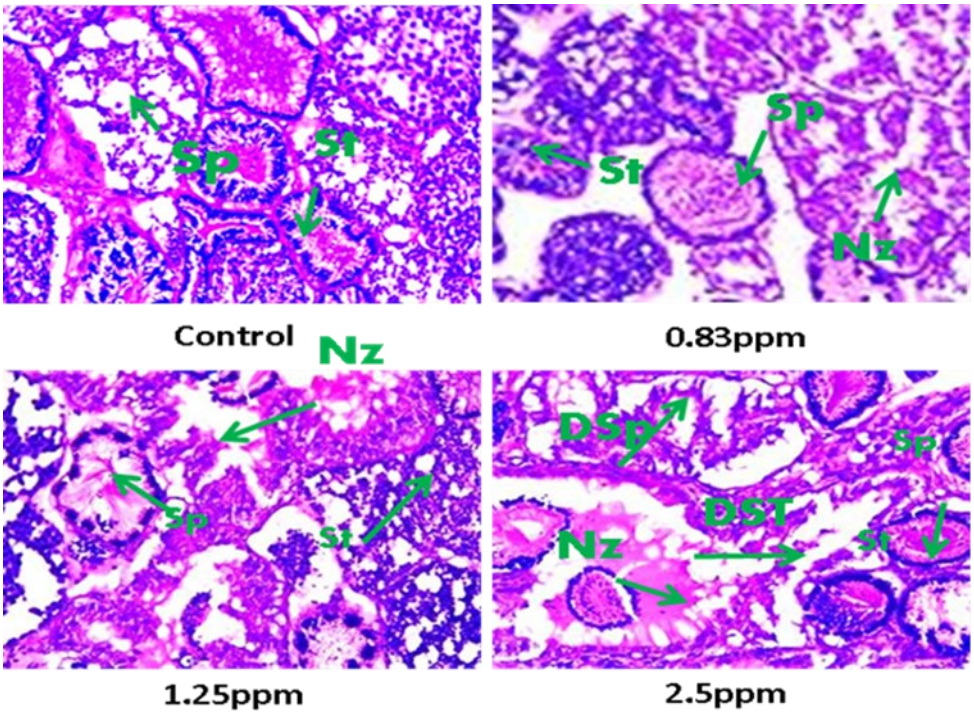

Fig. 11. *Impact of atrazine on the testis of Danio rerio. Control testicular sections showed normal testicular architecture with intact spermatogenic cells (Sp) and seminiferous tubules (St). Atrazine-treated groups showed progressive histopathological alterations, including nuclear shrinkage (Nz), disrupted spermatogenic cells (DSp), and damaged seminiferous tubules (DST), as indicated by green arrow mark.*

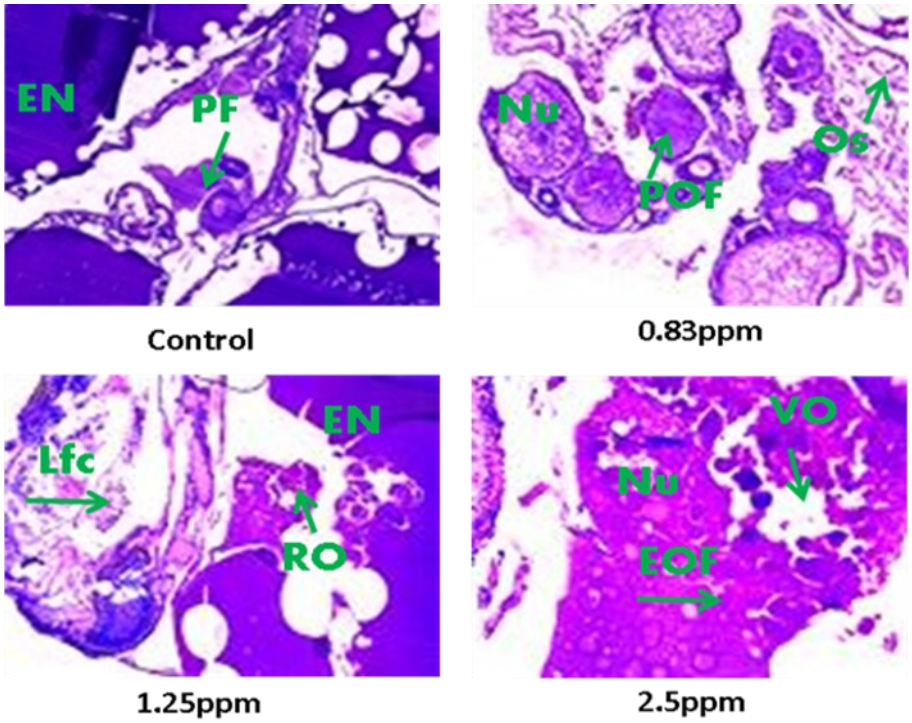

Fig. 12. *Impact of Atrazine on the Ovary of Danio rerio. Control ovary sections showed normal ovarian architecture with intact perinucleolar follicles (PF), normal ovarian stroma (OS), and egg nucleus (EN). Atrazine-treated groups showed progressive histopathological alterations, including post-ovulatory follicles (POF), ruptured oocytes (RO), vacuolated oocytes (VO), early oocyte follicles (EOF), nuclear changes (Nu), and loss of follicular cells (Lfc), as indicated by green arrow marks*

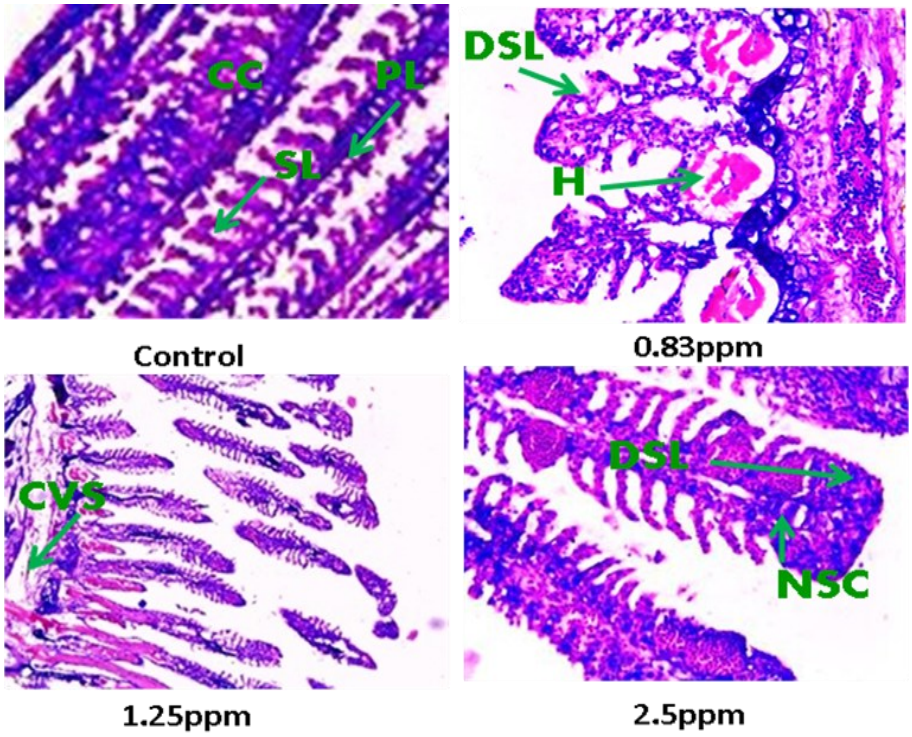

Fig. 13. *Impact of atrazine on the gill of Male Danio rerio. Control gill sections showed normal gill architecture with intact cartilage core (CC), primary lamellae (PL), and secondary lamellae (SL). Atrazine-treated groups showed progressive histopathological alterations, including distorted secondary lamellae (DSL), hyperplasia (H), congestion of the vascular space (CVS), and necrosis of secondary gill cells (NSC), as indicated by green arrowheads*

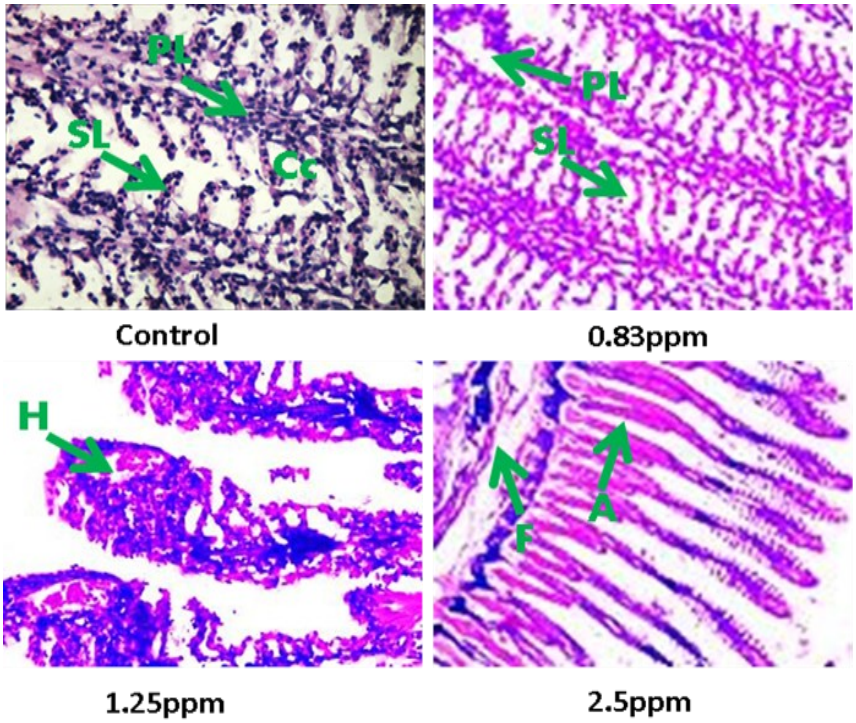

Fig. 14. *Impact of atrazine on the gill of female Danio rerio. Control gill sections showed normal gill architecture with intact primary lamellae (PL), secondary lamellae (SL), and cartilage core (Cc). Atrazine-treated groups (three concentrations) showed progressive histopathological alterations, including hyperplasia (H), fusion of lamellae (F), and aneurysm (A), as indicated by green arrow marks*

to atrazine. Although reduced G6PDH activity in treated *Danio rerio* indicates a potential alteration in testicular metabolic activity, it does not provide definitive evidence of impaired interstitial (Leydig) cell function. Because G6PDH plays a key role in maintaining cellular redox balance through the pentose phosphate pathway, its suppression may reflect generalized metabolic stress rather than a specific disruption of steroidogenic processes. To establish a direct mechanistic link between atrazine exposure and interstitial cell impairment, future studies incorporating testosterone quantification and Leydig cell–specific functional markers are required (Fujii, 2024).

Histological analysis of *Danio rerio* exposed to atrazine revealed concentration-dependent toxicity affecting multiple organs. In the gonads, atrazine exposure resulted in testicular atrophy, reduced testis weight, degeneration of the seminiferous epithelium, necrosis, and disorganized gonadal architecture. The liver exhibited pronounced pathological changes, including cloudy swelling of hepatocytes, pycnotic nuclei, lipoid vacuolation, and focal necrosis, findings consistent with previous reports of pesticide-induced hepatic damage in other fish species (Figs. 9 and 10) (Jesna *et al.,* 2024). The gills, identified as the primary target tissue, developed progressive lesions, including epithelial hyperplasia, lamellar fusion, increased epidermal thickness, aneurysmal dilations, and necrosis, which are likely to impair respiration and osmoregulation. Comparable degenerative changes were observed in the ovary and additional organs, indicating systemic toxicity. Collectively, these results demonstrate that atrazine exposure causes significant structural damage in zebrafish tissues, suggesting widespread physiological disruption and underscoring the herbicide's potential to compromise metabolic, reproductive, and respiratory functions in aquatic organisms (Maia *et al.,* 2025). Future studies should build on these findings by investigating the molecular mechanisms underlying atrazine-induced toxicity, with emphasis on oxidative stress pathways, endocrine disruption, regulation of apoptosis, and gene expression associated with tissue injury and repair. Analysis of gene-expression changes in stress responses, detoxification pathways, and reproductive hormones will yield a more comprehensive understanding of atrazine's effects on zebrafish. These mechanistic insights will clarify the pathways through which atrazine mediates toxicity and inform the development of more robust frameworks for environmental risk assessment.

Exposure to atrazine in the present study resulted in a female-biased sex ratio and significant histopathological changes in the gonads of *Danio rerio*. These findings align with previously reported endocrine-disrupting effects in aquatic species. Atrazine interferes with steroidogenic pathways, primarily by upregulating aromatase (Cyp19a1a), the enzyme that converts andro-

gens to estrogens. Increased aromatase activity elevates circulating estrogen levels, thereby altering hormonal balance, promoting ovarian differentiation, and suppressing testicular development (Samardzija *et al.,* 2016). Furthermore, atrazine modulates hormone receptor signaling, including estrogen and androgen receptors, thereby disrupting normal endocrine regulation during the critical period of gonadal differentiation. These mechanisms likely account for the observed feminization and gonadal tissue damage (Carvalho *et al.,* 2025). Assessing the expression of key genes involved in sex differentiation and hormone synthesis, such as aromatase, estrogen receptors, and androgen receptors, would provide further mechanistic insight. Although gene expression analysis was not conducted in the present study, the findings indicate that early atrazine exposure can disrupt steroidogenic and hormone-responsive pathways (Figs. 11 and 12) (Wu *et al.,* 2025). Future research should incorporate molecular endpoints, including steroidogenic enzyme expression, receptor regulation, and endocrine-responsive gene networks, to elucidate the mechanisms underlying gonadal alterations and to improve ecological risk assessments of atrazine in fish populations.

Conclusion

In conclusion, this study demonstrated that sub-lethal exposure to the herbicide atrazine significantly impairs the physiological, biochemical, and histopathological health of *Danio rerio*. During a 100-day exposure period, zebrafish subjected to increasing concentrations of atrazine (0.83, 1.25, and 2.5 ppm) showed a dose-dependent reduction in growth, disturbances in enzymatic balance, and progressive tissue damage. Elevated levels of GOT and GPT, which serve as biomarkers of hepatic stress and cellular injury, indicate severe liver dysfunction and underscore the liver's susceptibility as the primary site of xenobiotic metabolism. Histopathological analysis revealed distinct organ-specific toxicity. Liver samples exhibited hepatocyte vacuolation, necrosis, and structural disruption, consistent with chronic chemical exposure. Gill tissues showed lamellar fusion, epithelial lifting, and vascular congestion, likely hindering gas exchange and disrupting osmoregulation. Degenerative changes in both testicular and ovarian tissues suggest that prolonged atrazine exposure may impair reproductive capacity. Collectively, these findings underscore the complex and systemic toxic effects of atrazine and support the use of *Danio rerio* as a sensitive indicator species in ecotoxicological studies. The evidence presented highlights the necessity for stricter herbicide regulation, improved monitoring of freshwater systems, and the implementation of sustainable agricultural practices to minimize chemical runoff. Further research should investigate multigenerational impacts, the potential for recovery after exposure ends, and the molecular mechanisms underlying these effects to better assess the long-term ecological risks associated with atrazine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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