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The effect of abamectin exposure on gametogenesis in zebrafish

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Today, pesticides are widely used to enhance agricultural yield mixed with soil and water, creating significant environmental pollution. The extensive use of insecticides for pest control has made this issue more pronounced. Abamectin, a key member of the avermectin family, is used as an insecticide and an antihelminthic agent in agriculture. It is an active and effective agricultural pesticide, particularly preferred for combating pests such as aphids and red spider mites. The dosage and frequency of its use vary depending on the target plant species and pest organism. For example, abamectin-based products with an 18 g/L EC formulation in apple orchards are typically recommended at 10 ml/100 L of water to control red spider mites. Although significantly below agricultural application levels, the low dose of 0.75 µg L^{-1} used in our study has demonstrated effects that cannot be overlooked. However, it can contaminate aquatic environments, posing harmful effects on organisms. Studies indicate that abamectin exposure may lead to serious health issues, showing toxic and reproductive toxicity effects in aquatic species. Examining abamectin's effects on testicular tissue revealed hypertrophy of Sertoli cells in the group exposed to 0.75 µg L^{-1} of abamectin. Apoptotic cells were observed in the groups exposed to 0.75 µg L^{-1} and 1.5 µg L^{-1}. At the same time, pyknotic structures, disruption of seminiferous tubules, interstitial fibrosis, and atrophic appearance were identified across all dose groups, with severity increasing dose-dependently. Analysis of ovarian tissue demonstrated distortion of the zona radiata in groups exposed to 0.75 µg L^{-1} and 1.5 µg L^{-1} of abamectin. Moreover, in all dose groups, thickening of the zona radiata, vacuolization, formation of degenerated follicles, and nuclear disruption were observed, with these pathological alterations exacerbating in a dose-dependent manner. Like many studies involving zebrafish, this research is crucial for assessing potential toxic effects that may pose risks to human health. This study examined the histopathological effects of varying doses of abamectin (0.75 µg L^{-1}, 1.5 µg L^{-1}, and 3 µg L^{-1}) on zebrafish gonads after 96 h of exposure. Using standard histological techniques, the samples prepared were stained with H&E and observed under a light microscope. Statistical analyses were conducted using SPSS 23. The normality of the data was assessed with the Shapiro–Wilk test. One-way ANOVA and Tukey post-hoc tests were used for normally distributed groups, while the Kruskal–Wallis and Dunnett's T3 tests were applied for non-normally distributed groups. All analyses were performed with a 95% confidence interval and a significance level of $p > 0.05$.

Keywords Abamectin, Toxicity, Histopathology, Gonad, Zebrafish

Avermectins, first obtained in 1967 through the fermentation of the soil-dwelling actinomycete *Streptomyces avermitilis*, are disaccharides carrying a 16-membered macrocyclic lactone ring[1,2]. Among the compounds belonging to the avermectin family—doramectin, ivermectin, abamectin, and moxidectin—abamectin and ivermectin are the most preferred[1].

Abamectin is a pesticide of the avermectin class, containing 80% avermectin B1a and 20% avermectin B1b. It acts on GABA receptors and possesses insecticidal, acaricidal, and antiparasitic properties[3–8]. With antihelminthic activity, this chemical is widely used globally[9]. Due to its use in various animals, it can enter aquatic environments through different pathways and negatively affect aquatic organisms as a pollutant[10]. Abamectin can degrade quickly when exposed to light, water, and soil. Its short lifespan makes it difficult to detect in aquatic environments[1,11]. The known soil metabolites of abamectin have been identified as 4-oxo-avermectin B1a, 8-carboxy-4-hydroxy avermectin B1a, 8a-hydroxyavermectin B1a, 8a-hydroxyavermectin B1a, 4,8a-dihydroxy-avermectin B1a, and 8a-oxo-4-hydroxy-avermectin B1a. The known groundwater metabolites have been identified as 8a-hydroxyavermectin B1a, 8a-oxo-avermectin B1a, 4-oxo-avermectin B1a, 4,8a-dihydroxy-avermectin B1a, and 8-carboxy-4-hydroxy avermectin B1a. Among these, the metabolites 4-oxo-avermectin

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B1a, 8a-hydroxyavermectin B1a, and 8a-hydroxyavermectin B1a have been reported to possess toxicity that may cause environmental pollution and should be evaluated for their potential contamination risk to water bodies[12].

The GABA receptors on which abamectin acts are in the central nervous system. GABA is a neurotransmitter that plays an essential role in the interaction between nerve and muscle cells. Unlike mammals, this chemical cross the blood–brain barrier in fish, leading to toxicity[13–16]. Additionally, studies on mice and dogs indicate that when abamectin is ingested orally, it is highly absorbed in the gastrointestinal tract and distributed to critical organs and tissues before being excreted from the body[17].

In recent years, reproductive toxicity, often caused by environmental pollutants and chemocrine-disrupting chemicals, has become a frequent issue, resulting in a reduction of reproductive potential due to structural deformities in reproductive cells[18,19]. Environmental pollutants disrupt the reproductive system by causing oxidative stress, structural anomalies, cell death, and impaired fertility. Endocrine-disrupting substances mimic or block hormones like estrogen and androgen, threatening species survival[20,21].

Over the last twenty years, research has shown that zebrafish have become a preferred model organism due to their ease of care, short lifespan, transparent embryos, ease of detecting behavioral changes, and numerous advantages in drug, toxicity, and disease treatment research. They are also helpful for genetic screening studies[22–24]. Compared to other rodent and mammal model organisms, zebrafish offer advantages such as ease of manipulation in disease modeling, greater resilience to environmental conditions, and lower costs[25–30]. Zebrafish are also widely used in toxicological assessments of xenobiotic substances and environmental pollutants, particularly in target organs. Additionally, zebrafish are a leading aquatic organism for research into gonadal toxicity[31–37]. Various studies have also demonstrated abamectin's adverse effects on zebrafish. It has been reported that abamectin delays bone mineralization in zebrafish larvae, leads to differential expression of genes related to bone development, increases mortality rates with rising doses, and triggers apoptosis (Wang et al. 2025[38]). Furthermore, it has been reported that abamectin exposure causes behavioral differences in movement and food search in zebrafish, leads to changes in body structure, and results in morphological alterations in hepatocytes, glycogen accumulation, degeneration, disorganization of the cytoplasm, and the formation of pyknotic nuclei[39].

This study aims to examine the structural effects of abamectin on zebrafish gonads at the light microscopy level (Fig. 1).

Material and methods

Chemicals

The abamectin (Agrimec® 18 EC, Bayer) used in our study, obtained from a local agricultural supplier, is a natural insecticide isolated from the fermentation of the soil microorganism *Streptomyces avermitilis*. The selection of exposure concentrations is based on the literature data from Novelli et al.[7].

Experimental animals

6-month-old, sexually mature young zebrafish were selected. Younger individuals were preferred as ovarian and testicular quality deteriorates with age. The 40 zebrafish used in this research were bred at the Istanbul University Sapanca Aquatic Organisms Experimental Unit and were obtained at the age of 6 months.

The adult zebrafish used in the experiment (20 females, 20 males) were obtained from the Istanbul University Sapanca Aquatic Organism Experiment Unit. They were bred at the facility where the experiment was conducted and randomly distributed in the aquariums. Each aquarium was stocked with 10 fish, consisting of 5 males

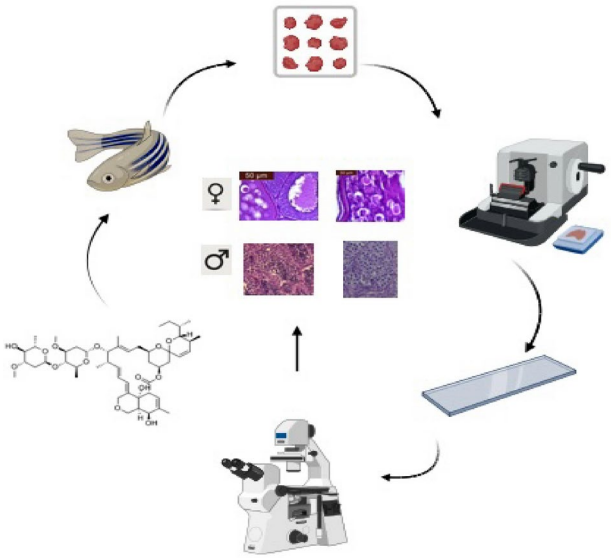

Fig. 1. Graphical Abstract. A short summary of the study titled "The Effect of Abamectin Exposure On Gametogenesis In Zebrafish".

and 5 females. The zebrafish were housed in 30 L aquariums under a 12-h light and 12-h dark photoperiod at a temperature of 28 ± 1 °C and a pH of 7.0 ± 0.5. The fish were fed Tetra© Pro Energy (Tetra Werke, Germany) twice daily and approximately 1% of their body weight. The water in the experimental tank was not renewed. The fish were exposed to abamectin, and the setup was left undisturbed for 96 h. Feces and food residues were removed daily during regular inspections.

Abamectin exposure

The zebrafish used in the experiment were divided into one control group and three experimental groups (n = 10). While no treatment was applied to the zebrafish in the control group, the fish in the experimental groups were exposed to different doses of abamectin (0.75 μgL^{-1}, 1.5 μgL^{-1}, 3 μgL^{-1}) for 5 days. Before the application, abamectin was dissolved in distilled water to prepare a stock solution. Then, it was administered to each aquarium by injecting appropriate concentrations using a micropipette. The fish, exposed to abamectin only once, remained in the same water for 96 h.

The 96-h LC_{50} concentration of abamectin in zebrafish (59 $\mu g\ L^{-1}$) was determined based on the study by Novelli et al.[7]. The LC_{50} dose of the chemical substance used in this study is known. Therefore, the abamectin concentrations to be applied to the experimental groups were decided to be 50%, 25%, and 12.5% of the determined dose, ensuring that they remained below the LC_{50} value, and the experiments were conducted accordingly.

Histological analysis

At the end of the experiment, the zebrafish were anesthetized with 250 mgL^{-1} tricaine methanesulfonate (MS-222; Sigma Aldrich). After dissection, the gonad tissues (ovaries and testes) were collected and fixed in Bouin's solution (for 24 h). Routine light microscopy procedures were applied to the gonad tissues[40]. The tissues were dehydrated with a graded ethanol series (70–100%), cleared with xylene, and embedded in paraffin at 58 °C. After the paraffin solidified, the tissue blocks were removed from their molds. Sections of 5 µm thickness were taken using a manual microtome (Leica RM2125RT). The sections were stained with hematoxylin and eosin (H&E), examined under a light microscope (Leica DM 500), and imaged with a Leica MC170 HD camera[41,42]. Additionally, examinations were performed under a light microscope during the staining process to check the staining quality.

Collection of histopathological data

Using a blinded technique, five randomly selected images of testes and five ovaries were scanned for histopathological damage. In the ovary preparations, increased oocyte atresia, decreased vitellogenesis, thickening of the zona radiata, fusion in cortical alveoli, vacuolization in the ooplasm, and membrane invaginations were photographed and counted. In the testis preparations, fusion in the seminiferous tubules, a decrease in spermatogonia, vacuolization in the seminiferous tubules, disruption in the integrity of spermatogenic cell clusters, and vacuolization in the interstitial tissue were quantitatively examined. Cells were counted in a specific area under a light microscope and quantitatively evaluated. The counts were performed randomly and repeated five times[43]. The counting results are presented as mean ± standard deviation.

Statistical analysis

Statistical analyses were performed using SPSS 23 software. The normality of data distribution was examined with the Shapiro–Wilk test. One-way ANOVA was used to compare groups with normal distribution. For groups that did not show a normal distribution, the Kruskal–Wallis test was applied. The Tukey posthoc test was used for normally distributed data, and Dunnett's T3 test was applied for non-normally distributed data. All analyses were conducted with a 95% confidence interval and a significance value of $p > 0.05$. For statistical analysis, 5 fields were counted from each slide, and 5 slides were counted for each dose. A total of 25 counts were performed for each dose.

Ethical statement

All procedures and experiments complied with the ARRIVE guidelines and the Istanbul University Local Ethics Committee for Animal Experiments (HADYEK), which approved the experimental setup and fish care on 10.04.2023, with the ethical approval number 2023/12. in compliance with the fish acute test followed the OECD guideline 203.

Results

Effect of abamectin on ovary tissue

The histopathological changes observed in the zebrafish ovary tissue were compared for four different abamectin concentrations (0.75 μgL^{-1}, 1.5 μgL^{-1}, 3 μgL^{-1}) and the control group (Figs. 2, 3). The histological examination of the zebrafish control group's ovarian tissue following Hematoxylin and Eosin (H&E) staining revealed a standard histological structure (Fig. 4A, B, C). Histopathological examination revealed that exposure to 0.75 $\mu g\ L^{-1}$ Abamectin increased the number of primary oocytes in ovarian tissue. However, a concurrent rise in the rate of vacuolization was observed. Separating the zona radiata and follicular epithelial cells was evident (Fig. 4A). Regions within the membrane exhibited both unfolding and folding. Cortical alveoli displayed structural disintegration and dispersion, leading to prominent atretic oocyte formation.) developed in the interstitial areas between oocytes (Fig. 5A, B). Thickening of the zona radiata is also observed (Figure 5C).

Histopathological changes in zebrafish ovarian tissue were more pronounced at a 1.5 $\mu g\ L^{-1}$ Abamectin exposure level. Disruption in cortical alveoli and separations between the zona radiata and ooplasm were identified (Fig. 6A). Enlargement of interstitial spaces, folds, and undulations in the zona radiata region was

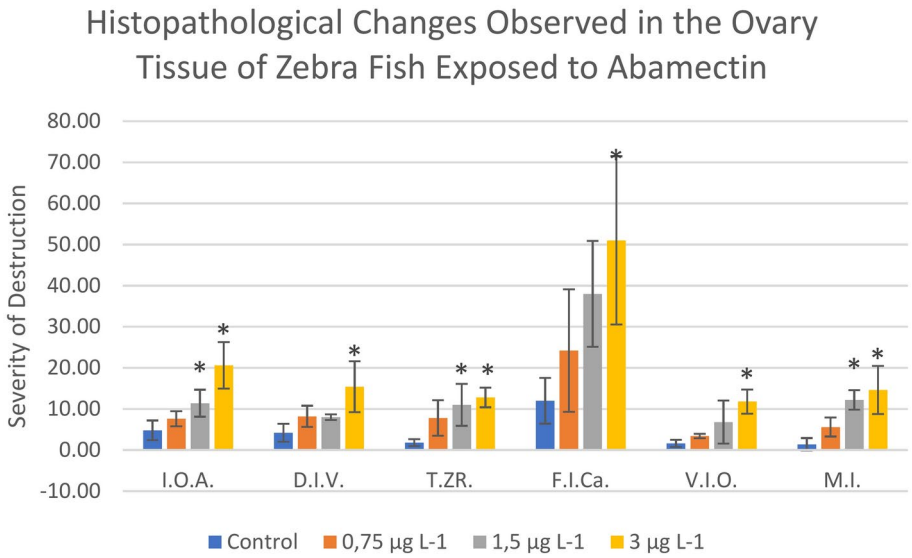

Fig. 2. Graph of Histopathological Changes Observed in the Ovary Tissue of Zebrafish Exposed to Abamectin. (*): Significant differences ($p > 0.05$), I.O.A.: Increased Oocyte Atresia, D.I.V.: Decrease in Vitellogenesis, T.ZR.: Thickening of Zona Radiata, F.I.Ca.: Fusion in Cortical Alveoli, V.I.O.: Vacuolization in Oolasm, M.I.: Membrane Invagination.

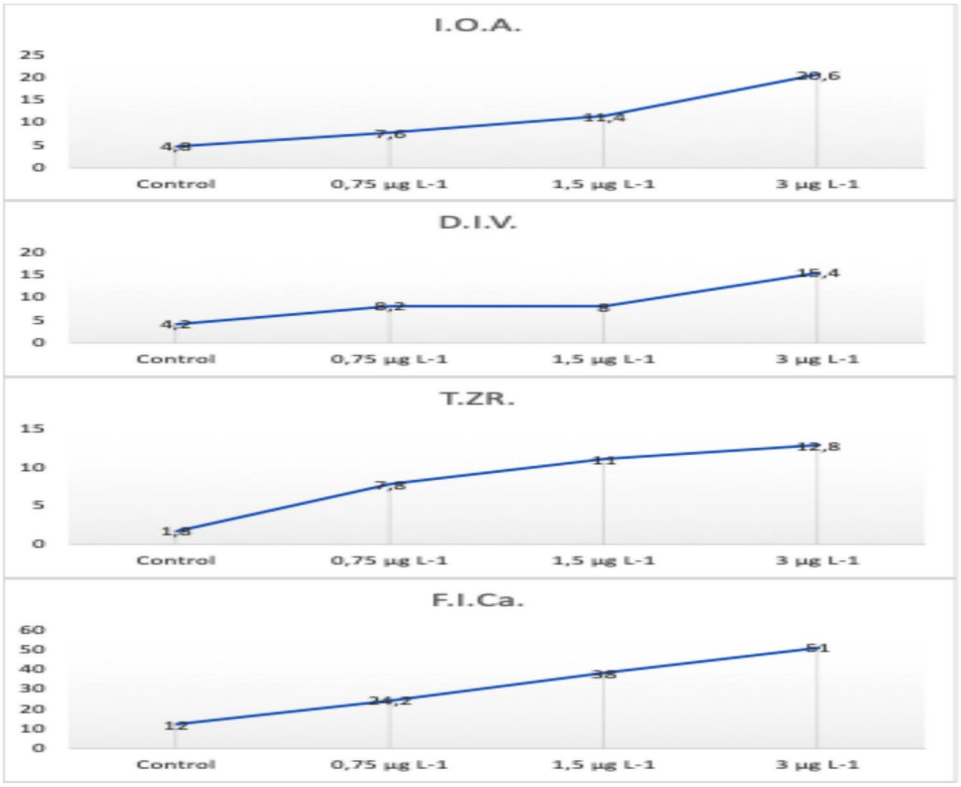

Fig. 3. Dose-dependent histopathological changes in zebrafish ovary after abamectin exposure. I.O.A.: Increased Oocyte Atresia, D.I.V.: Decrease in Vitellogenesis, T.ZR.: Thickening of Zona Radiata, F.I.Ca.: Fusion in Cortical Alveoli, V.I.O.: Vacuolization in Ooplasm, M.I.: Membrane Invagination.

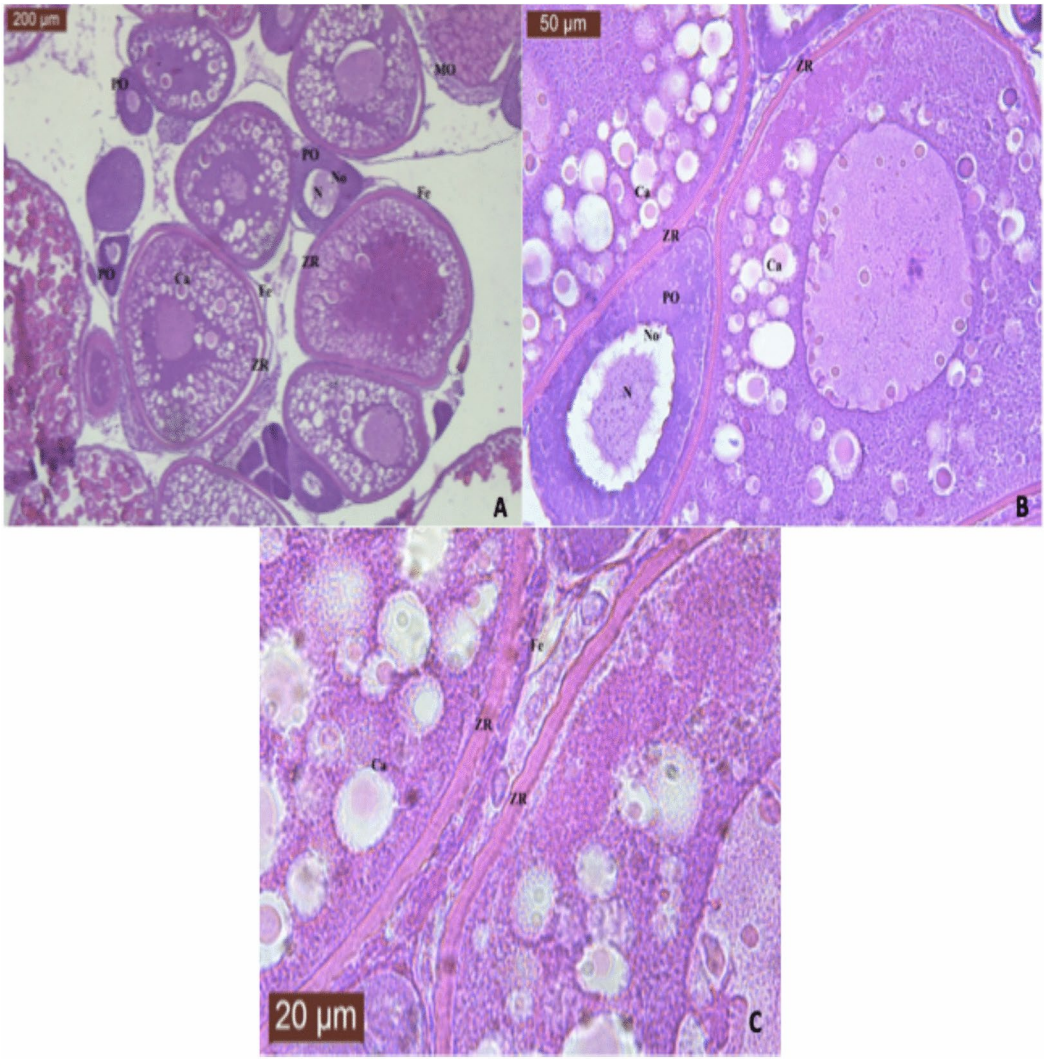

Fig. 4. Ovarian tissue of the control group in zebrafish. **A** ×10 magnification, H&E. **B** ×40 magnification, H&E. **C** ×100 magnification, H&E. Primary Oocyte (PO), Cortical Oocyte (CO), Vitellogenic Oocyte (VO), Mature Oocyte (MO), Cortical Alveolus (Ca), Nucleus (N), Nucleolus (No), Follicular Epithelium (Fe), Zona Radiata (ZR).

observed. Certain oocytes exhibited reduced vitellogenesis, and atretic follicle formation was apparent (Fig. 6B, C).

Ovarian tissue exposed to 3 µg L^{-1} Abamectin demonstrated dose-dependent exacerbation of histopathological alterations. Vacuolization in the ooplasm and degeneration of follicular structures were detected (Fig. 7A, B). Some oocytes exhibited thickening in the zona radiata, while others displayed increased vacuolization (Fig. 7A). Additionally, folds in the zona radiata were identified. Hypertrophy and structural disruption in cortical alveoli were documented, accompanied by nuclear shrinkage and nuclear degradation (Fig. 7C, D).

When we statistically analyzed the data (Table 1), a low value (4.80 ± 2.39) for increased oocyte atresia was observed in the control group. While significant increases were seen in the 0.75 µgL^{-}1 and 1.5 µgL^{-1} groups, a marked increase and significant difference (*) was noted in the 3 µgL^{-1} group (20.60 ± 5.64). This indicates that higher doses of abamectin cause a severe increase in oocyte atresia.

In the evaluation of decreased vitellogenesis, a low level of vitellogenesis was observed in the control group (4.20 ± 2.17), and no significant difference was found between the 0.75 µgL^{-1} and 1.5 µgL^{-1} groups. However, in the 3 µgL^{-1} group (15.40 ± 6.19), a significant increase (*) was noted, indicating that exposure to high doses of abamectin significantly reduces vitellogenesis. Regarding the thickening of the zona radiata, a low level of thickening was observed in the control group (1.80 ± 0.84). Significant increases (*) were seen in the 1.5 µgL^{-1} and 3 µgL^{-1} groups, showing a significant difference in zona radiata thickening with increasing abamectin concentrations.

In examining fusion in cortical alveoli, a relatively low value (12.00 ± 5.57) was noted in the control group. Significant increases (*) were observed in the 1.5 µgL^{-1} and 3 µgL^{-1} groups (38.00 ± 12.88 and 51.00 ± 20.47), indicating that the rate of fusion in cortical alveoli increases with the dose of abamectin. In evaluating

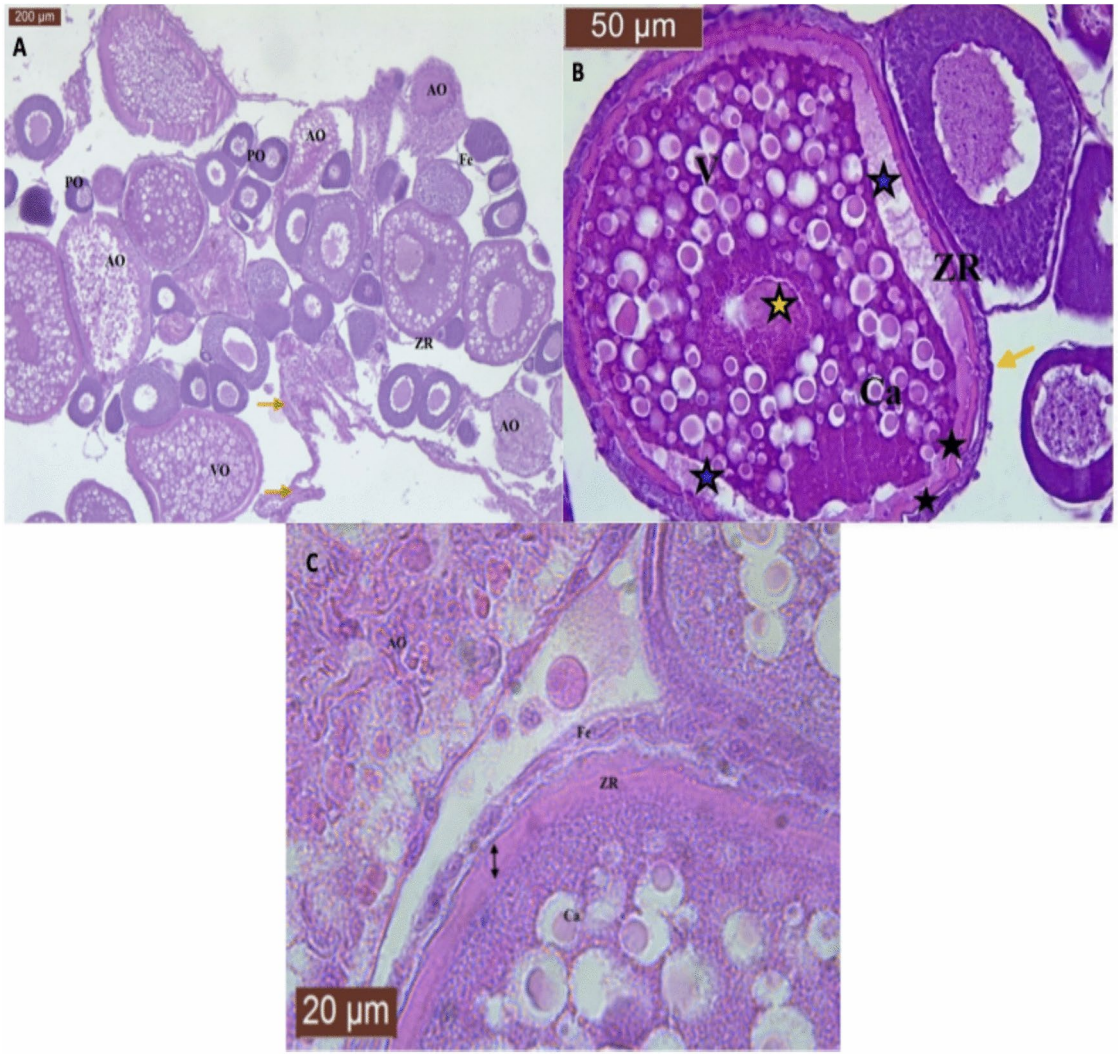

Fig. 5. Ovarian tissue exposed to 0.75 µgL^{-1} abamectin. **A** ×10 magnification, H&E. **B** Cortical alveolar oocyte showing early signs of atresia. ×40 magnification, H&E. **C** Thickened Zona Radiata. ×100 magnification, H&E. Primary Oocyte (PO), Vitellogenic Oocyte (VO), Atretic Oocyte (AO), Follicular Epithelium (Fe), Cortical Alveolus (Ca), Deformed Zona Radiata (Black Arrow), Zona Radiata (ZR), Gap between ZR and Fe (Pink Star), Thickening of the Zona Radiata (double-headed black arrow), Fibrosis (Orange arrow), Cortical Alveolus (Ca), Distortion and irregular appearance in the Zona Radiata (Black Star), Gap between ooplasm and zona radiata (Blue Star), Nuclear Degeneration (Yellow Star).

vacuolization in the ooplasm, a low level of vacuolization was observed in the control group (1.60 ± 0.89). Significant differences (*) were found in the 1.5 µgL^{-1} and 3 µgL^{-1} groups, indicating that the abamectin concentration increases the ooplasm's vacuolization. In the assessment of membrane invagination, the lowest values were observed in the control group (1.40 ± 1.52). There was a significant increase (*) in the 1.5 µgL^{-1} and 3 µgL^{-1} groups. It appears that increasing doses of abamectin led to an increase in membrane invagination.

As the abamectin concentration increased, significant increases were observed in all histopathological parameters. Particularly at the 3 µgL^{-1} dose, significant differences (*) appeared in most parameters. These results indicate that abamectin causes histopathological changes in zebrafish ovaries and that these effects become more severe with increased dosage.

Post-hoc analyses, such as the Tukey test, were applied to determine significant differences between the parameters, and significant differences (*) were identified.

Effects of abamectin on testis tissue

Histopathological changes in the testis tissue of zebrafish exposed to abamectin were examined at four different doses, represented as "Control," "0.75 µgL^{-1}," "1.5 µgL^{-1}," and "3 µgL^{-1}" (Figs. 8, 9). Histological examination of the zebrafish control group testis tissue revealed seminiferous tubules surrounded by seminiferous epithelium. Oval-nucleated, round-shaped Leydig cells, identified as interstitial cells responsible for androgen synthesis, were observed in the interstitial area between the seminiferous tubules. Within the seminiferous tubules,

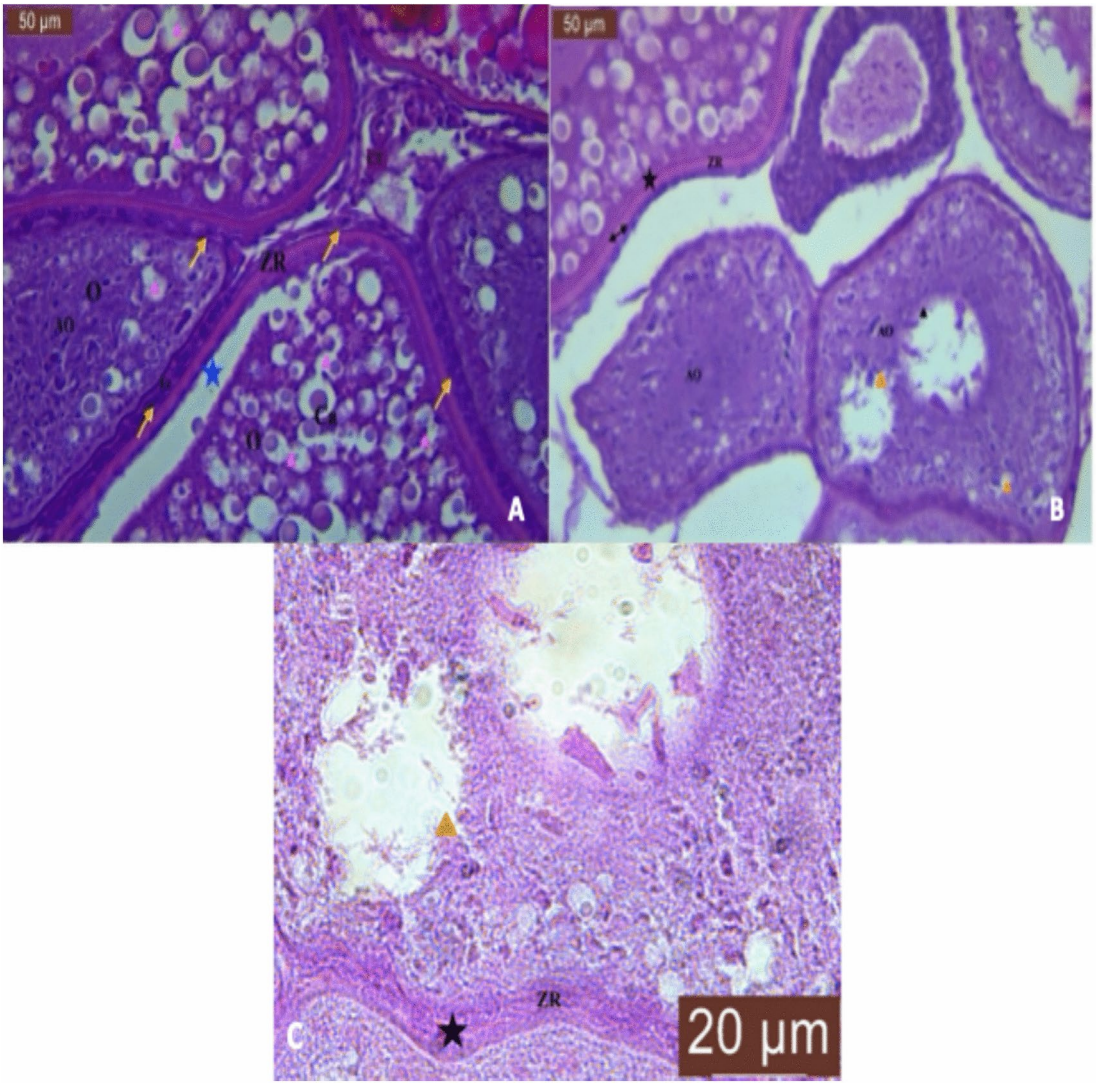

Fig. 6. Ovarian tissue exposed to 1.5 µgL^{-1} abamectin. **A** ×40 magnification, H&E. **B** ×40 magnification, H&E. **C** Atretic Oocyte. ×100 magnification, H&E. Atretic Oocyte (AO), Zona Radiata (ZR), Connective Tissue (CT), Ooplasm (O), Cortical Alveolus (Ca), Follicular Epithelium (yellow arrow), Disruption in the cortical alveolus (pink triangle), Gap between ooplasm and ZR (Blue Star), Primary Oocyte (PO), Thickening of the Zona Radiata (double-headed black arrow), Vacuolization (Orange Triangle), Distortion in the ZR (Black Star).

various spermatogenic cells were identified, including spermatogonia with large, prominent nuclei (organized in clusters of 2 or 3), primary spermatocytes characterized by smaller volume and dense cytoplasm compared to spermatogonia, secondary spermatocytes with a smaller and more rounded morphology than primary spermatocytes, spermatids observed as small cells within clusters and centrally located clusters of sperm cells within the tubules (Fig. 10). Localized vacuole formation was prominently observed in the testicular tissue of zebrafish exposed to 0.75 µg L^{-1} Abamectin. Increased connective tissue (interstitial fibrosis) was detected in the intertubular regions. Within the seminiferous tubules, a higher sperm density was observed, while adipocyte infiltration was sporadically identified in the testicular tissue (Fig. 11A, B). Pycnotic nucleus formation was noticeable within spermatogenic cell clusters, and a reduction in spermatid cells was observed. A decrease in developing spermatogenic cell clusters was also recorded. Additionally, increased vascularization became prominent in the interstitial area (Fig. 11C, D). Exposure to 1.5 µg L^{-1} Abamectin resulted in significant disruption of seminiferous tubule integrity compared to the control group. A marked reduction in the clusters of spermatogenic cells was observed within the seminiferous tubules, with some tubules showing no developing spermatogenic cell clusters. Necrotic areas and apoptotic cells were identified within these clusters. Hyperplasia was observed in Leydig cells and clusters of degenerated (structurally compromised) cells (Fig. 12A, B). Germinal epithelium disruption and loss of tubular integrity were noted with structural disintegration of cell clusters. Advanced vacuolization was prevalent throughout the testicular tissue, along with widespread atrophy (Fig. 12C). Testicular tissue exposed to 3 µg L^{-1} Abamectin exhibited severe histological lesions. These included pronounced thickening of tubule walls, increased atrophic cells within the tubules, pyknotic cells, and a dramatic

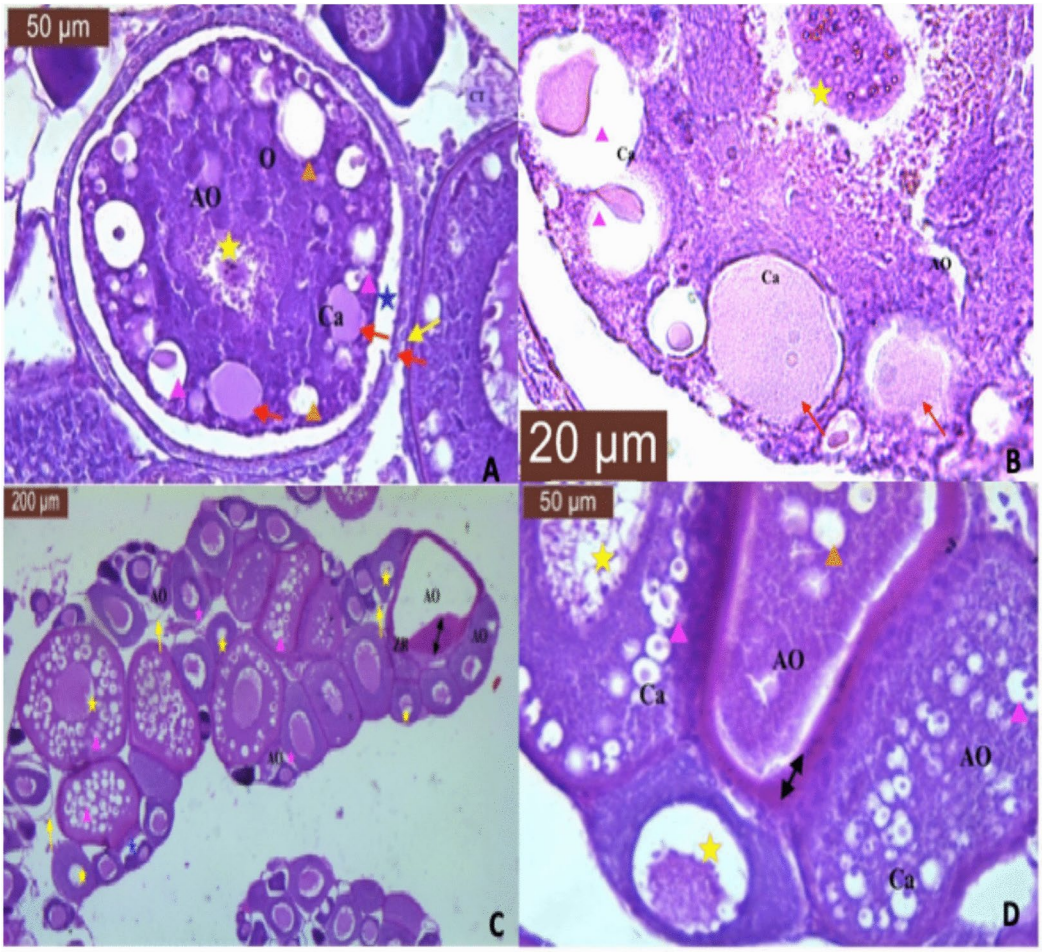

Fig. 7. Ovarian tissue exposed to 3 μgL^{-1} abamectin. **A** ×40 magnification, H&E. **B** ×100 magnification, H&E. **C** ×10 magnification, H&E. **D** ×40 magnification, H&E. Atretic Oocyte (AO), Ooplasm (O), Cortical Alveolus (Ca), Zona Radiata (ZR), Follicular Epithelium (yellow arrow), Nuclear Degeneration (Yellow Star), Hypertrophy in the cortical alveolus (Red Arrow), Gap between ooplasm and ZR (Blue Star), Disruption in the cortical alveolus (Pink Triangle), Thickening in the ZR (double-headed black arrow), Vacuolization in the cortical alveolus (Orange Triangle), Gap between ZR and Fe (Pink Star).

	Control	0,75 μgL^{-1}	1,5 μgL^{-1}	3 μgL^{-1}
I.O.A	$4,80 \pm 2,39^{a}$	$7,60 \pm 1,82^{ab}$	$11,40 \pm 3,29^{*b}$	$20,60 \pm 5,64^{*c}$
D.I.V	$4,20 \pm 2,17^{a}$	$8,20 \pm 2,59^{a}$	$8,00 \pm 0,71^{a}$	$15,40 \pm 6,19^{*b}$
T.ZR	$1,80 \pm 0,84^{a}$	$7,80 \pm 4,32^{ab}$	$11,00 \pm 5,10^{*b}$	$12,80 \pm 2,39^{*b}$
F.I.Ca	$12,00 \pm 5,57^{a}$	$24,20 \pm 14,91^{a}$	$38,00 \pm 12,88^{ab}$	$51,00 \pm 20,47^{*b}$
V.I.O	$1,60 \pm 0,89^{a}$	$3,40 \pm 0,55^{a}$	$6,80 \pm 5,22^{ab}$	$11,80 \pm 2,95^{*b}$
M.I	$1,40 \pm 1,52^{a}$	$5,60 \pm 2,30^{a}$	$12,20 \pm 2,39^{*b}$	$14,60 \pm 5,86^{*b}$

Table 1. Histopathological changes observed in the ovary tissue of zebrafish exposed to abamectin. (*): Significant differences ($p > 0.05$). (^{a},^{b},^{c}) : Dose groups with statistically significant differences according to the Tukey post-hoc test results. I.O.A.: Increased Oocyte Atresia, D.I.V.: Decrease in Vitellogenesis, T.ZR.: Thickening of Zona Radiata, F.I.Ca.: Fusion in Cortical Alveoli, V.I.O.: Vacuolization in Ooplasm, M.I.: Membrane Invagination.

loss of developing spermatogenic cell clusters. Advanced vacuolization and extensive fibrosis in the intertubular regions were prominent (Fig. 13A, B). A marked increase in testicular degeneration was identified, accompanied by the disruption of seminiferous tubule integrity, with no spermatogenic cell clusters observed within the seminiferous tubules (Fig. 13A).

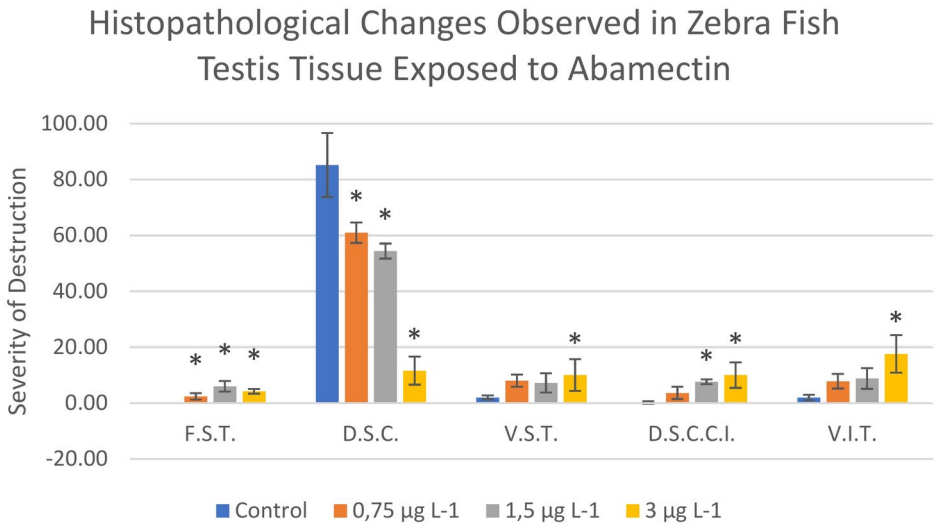

Fig. 8. Graph of Histopathological Changes Observed in Zebrafish Testis Tissue Exposed to Abamectin. (*): Significant differences ($p > 0.05$) F.S.T.: Fusion in Seminiferous Tubules D.S.C.: Decrease in Spermatogonium Count, V.S.T.: Vacuolization in Seminiferous Tubules, D.S.C.C.I.: Disruption of Spermatogenic Cell Cluster Integrity, V.I.T.: Vacuolization in Interstitial Tissue.

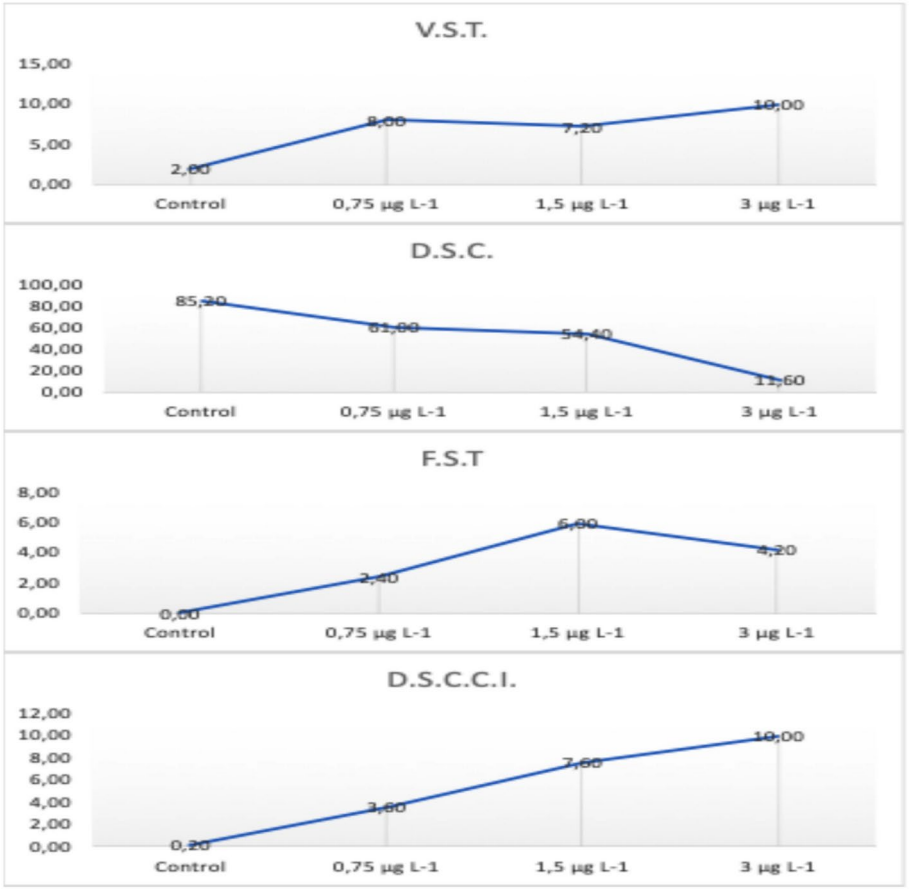

Fig. 9. Dose-dependent histopathological changes in zebrafish testis after abamectin exposure. F.S.T.: Fusion in Seminiferous Tubules, D.S.C.: Decrease in Spermatogonium Count, V.S.T.: Vacuolization in Seminiferous Tubules, D.S.C.C.I.: Disruption of Spermatogenic Cell Cluster Integrity, V.I.T.: Vacuolization in Interstitial Tissue.

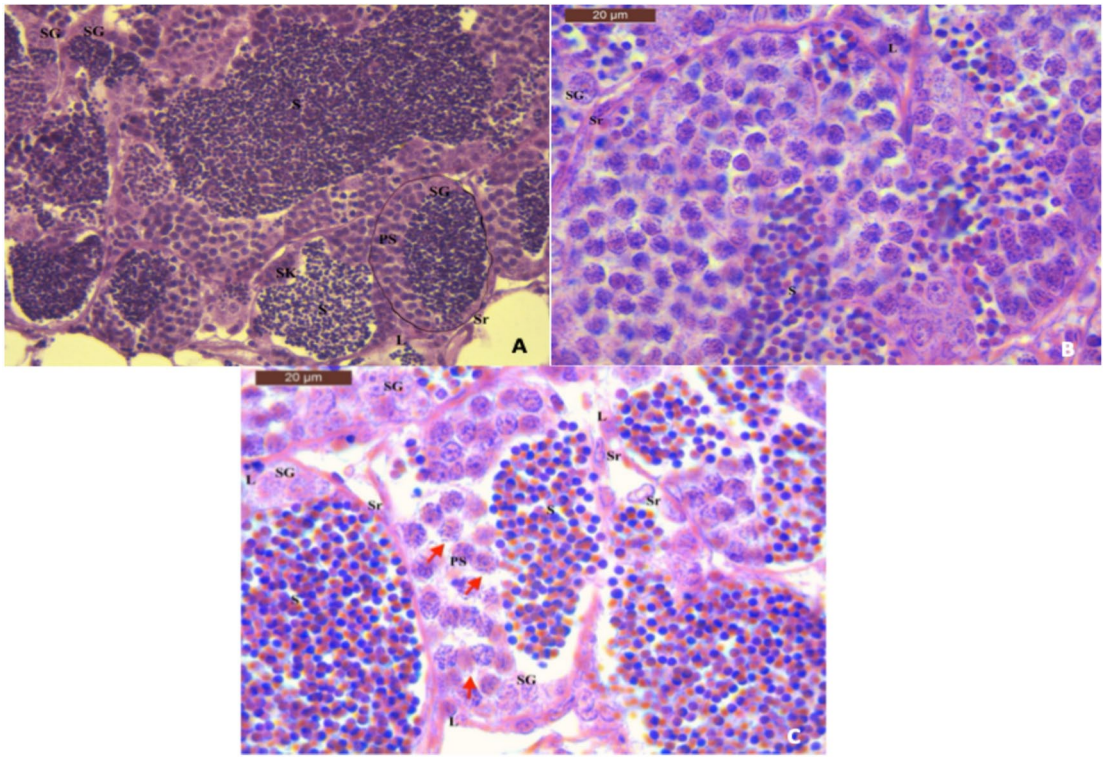

Fig. 10. Testicular tissue of the control group in zebrafish. **A** ×40 magnification, H&E. **B** ×100 magnification, H&E. **C** ×100 magnification, H&E. Sperm (S), Spermatogonium (SG), Primary Spermatocyte (PS), Secondary Spermatocyte (SK), Leydig Cell (L), Sertoli Cell (Sr), Hypertrophy in primary spermatocytes (red arrow).

Upon analyzing the data regarding histopathological changes observed in the testis tissue of zebrafish due to abamectin exposure (Table 2), no fusion was observed in the seminiferous tubules of the control group (0.00 ± 0.00). Significant increases in the fusion rate (*) were observed in groups with 0.75 μgL^{-1} and higher. Notably, fusion reached its highest level in the 1.5 μgL^{-1} group (6.00 ± 1.87), followed by a slight decrease in the 3 μgL^{-1} dose. As a result, it was observed that as the concentration of abamectin increased, so did the fusion in the seminiferous tubules.

When examining the decrease in the number of spermatogonia, it was found that the control group had a very high number of spermatogonia (85.20 ± 11.43). A significant decrease (*) was noted in the 0.75 μgL^{-1} and 1.5 μgL^{-1} doses, with the lowest value recorded in the 3 μgL^{-1} group (11.60 ± 5.03). This indicates that an increase in the dose of abamectin leads to a significant reduction in the number of spermatogonia.

In the control group, a low level of vacuolization in the seminiferous tubules was observed (2.00 ± 0.71). In the 3 μgL^{-1} group, a significant increase (*) in vacuolization was observed (10.00 ± 5.70), indicating that abamectin concentrations increase vacuolization.

Data regarding the integrity of spermatogenic cell clusters showed almost no disturbance in the control group (0.20 ± 0.45). With the increase in abamectin dose, a significant disturbance was observed, particularly in the 3 μgL^{-1} group (10.00 ± 4.58), indicating that high doses disrupt the integrity of spermatogenic cell clusters.

When examining the data on vacuolization in the interstitial tissue, a low level of vacuolization was observed in the control group (2.00 ± 1.00). It was noted that vacuolization significantly increased, especially in the 3 μgL^{-1} group (*) (17.60 ± 6.69), demonstrating that abamectin leads to vacuolization in the interstitial tissue.

Parameters such as fusion in seminiferous tubules (S.T.F.) and decreased spermatogonia (S.S.A.) showed significant differences, especially at doses of 0.75 μgL^{-1} and above. It was observed that as the dose increased, histopathological damage also increased. Vacuolization showed an increase in both the seminiferous tubules and the interstitial area, depending on the dose of abamectin. The disturbance in the integrity of spermatogenic cell clusters (S.H.K.B.B.) became pronounced at the highest dose of abamectin.

These results indicate that exposure to abamectin causes severe histopathological changes in the testis tissue of zebrafish and that these changes intensify with increasing doses.

Discussion

The various types of pesticides used for many purposes raise concerns due to their potential environmental impacts, even at low concentrations. One of the most widely used types of pesticides in agricultural fields worldwide is avermectins. Components of the avermectin family belong to a class of highly effective but toxic natural substances used as medications in various treatments for humans and animals, as well as for plant protection, with abamectin being one of the most used compounds in this family[1]. In addition to its agricultural

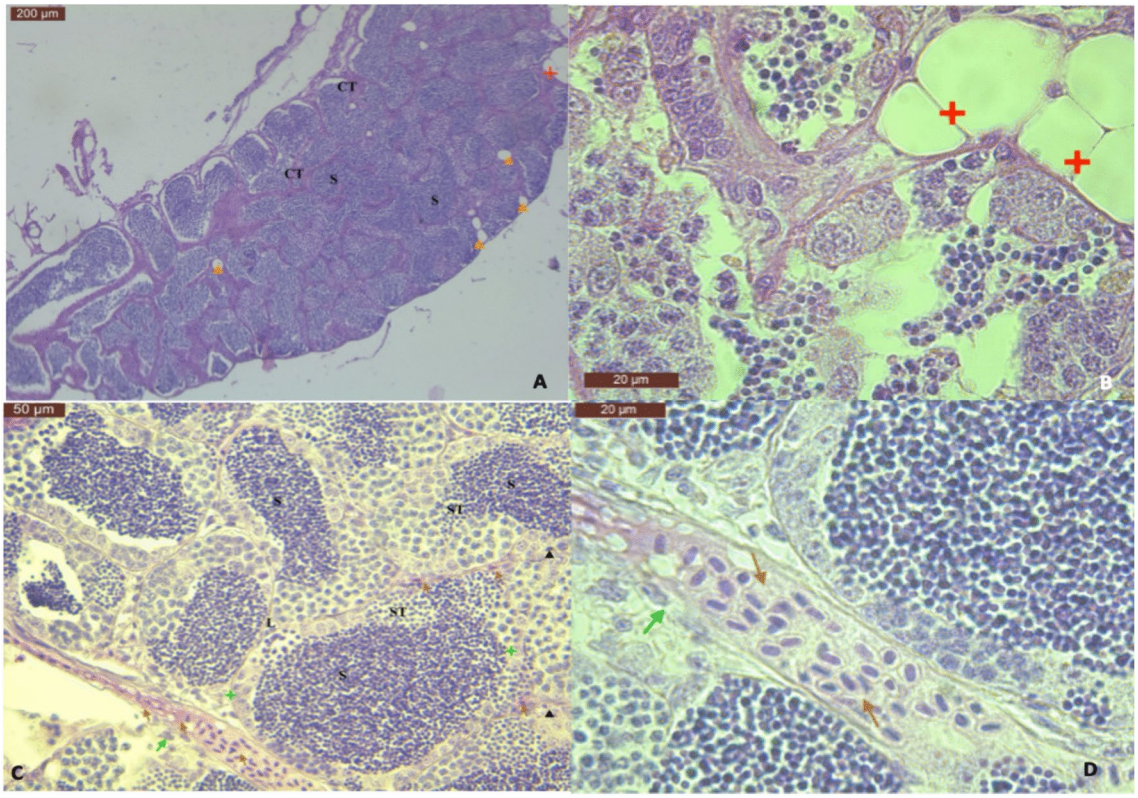

Fig. 11. Testicular tissue exposed to 0.75 µgL^{-1} abamectin. **A** ×10 magnification, H&E. **B** ×100 magnification, H&E. **C** ×40 magnification, H&E. **D** ×100 magnification, H&E. Sperm (S), Spermatid (ST), Vacuolization (Orange Triangle), Connective Tissue (CT), Adipocyte infiltration (Red Plus), Pyknotic Nucleus (Black Triangle), Vascularization (Brown Arrow), Degenerated Germ Cell Cluster (Green Arrow).

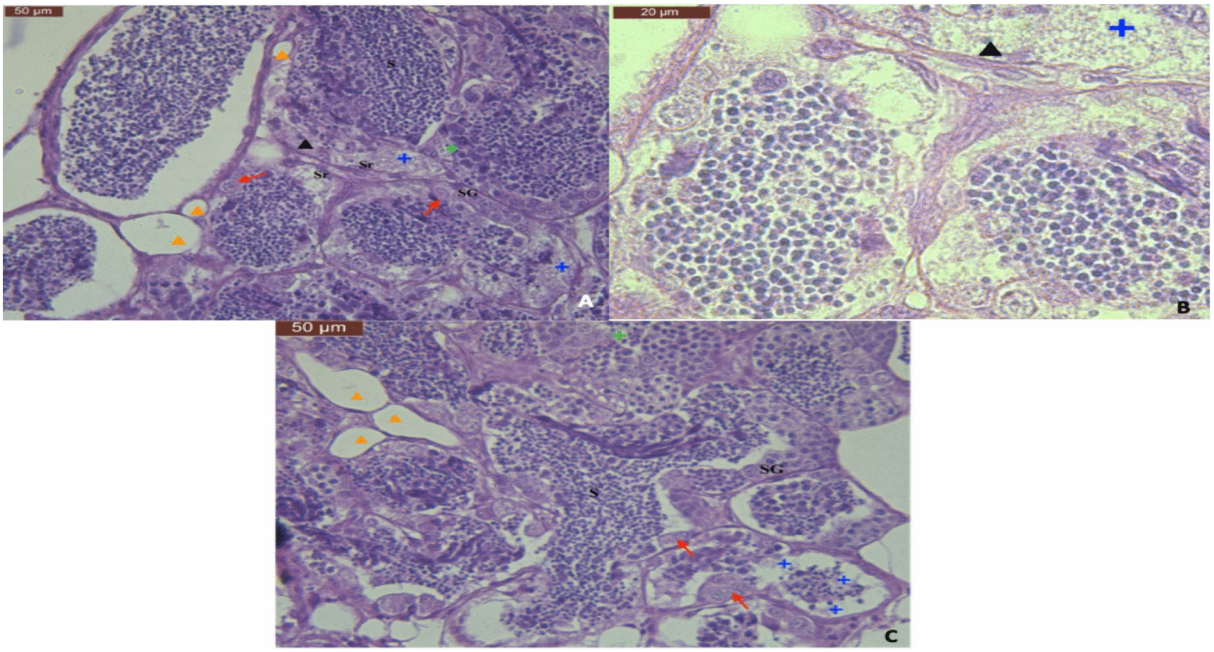

Fig. 12. Testicular tissue exposed to 1.5 µgL^{-1} abamectin. **A** ×40 magnification, H&E. **B** ×100 magnification, H&E. **C** ×40 magnification, H&E. Necrotic area (Blue Plus), Pyknotic Nucleus (Black Triangle), Apoptotic Cell (Green Plus), Sperm (S), Sertoli Cell (Sr), Hypertrophy in Spermatogonium (SG) Cells (Red Arrow), Vacuolization (Orange Arrow), Clusters of cells with disrupted structural integrity (Blue Plus).

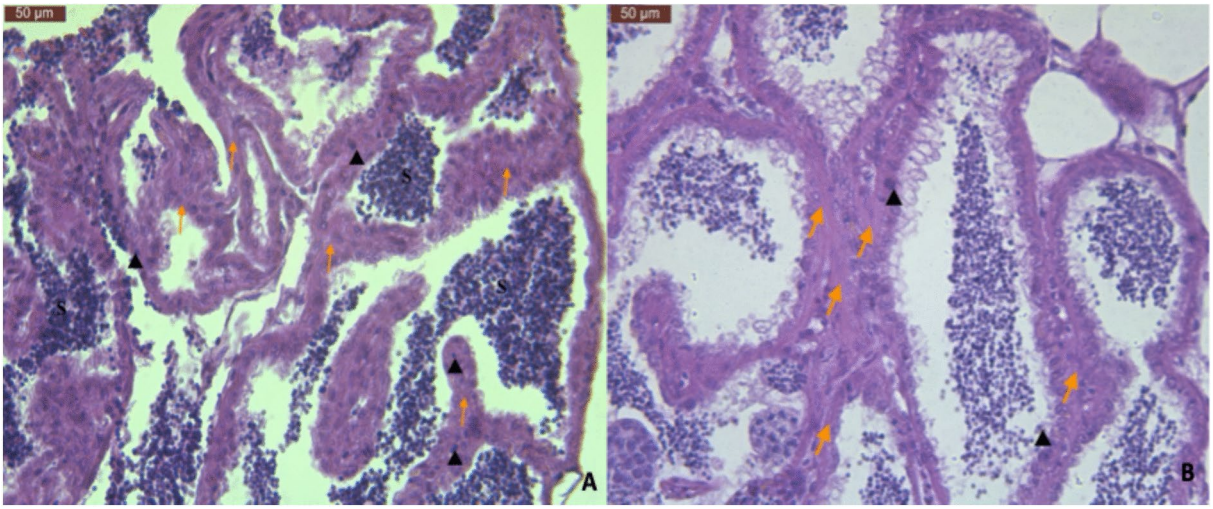

Fig. 13. Testicular tissue exposed to 3 μgL^{-1} abamectin. **A** Advanced atrophic appearance in testicular tissue. ×40 magnification, H&E. **B** ×40 magnification, H&E. Interstitial Fibrosis (Orange Arrow), Pyknotic appearance in increasing interstitial cells (Black Triangle).

	Control	0,75 μgL^{-1}	1,5 μgL^{-1}	3 μgL^{-1}
F.S.T	$0,00 \pm 0,00^{a}$	$2,40 \pm 1,14^{*b}$	$6,00 \pm 1,87^{*bc}$	$4,20 \pm 0,84^{*c}$
D.S.C	$85,20 \pm 11,43^{a}$	$61,00 \pm 3,67^{*b}$	$54,40 \pm 2,70^{*b}$	$11,60 \pm 5,03^{*c}$
V.S.T	$2,00 \pm 0,71^{a}$	$8,00 \pm 2,19^{ab}$	$7,20 \pm 3,42^{ab}$	$10,00 \pm 5,70^{*b}$
D.S.C.C.I	$0,20 \pm 0,45^{a}$	$3,60 \pm 2,19^{ab}$	$7,60 \pm 0,89^{*bc}$	$10,00 \pm 4,58^{*c}$
V.I.T	$2,00 \pm 1,00^{a}$	$7,80 \pm 2,59^{a}$	$8,80 \pm 3,70^{a}$	$17,60 \pm 6,69^{*b}$

Table 2. Histopathological changes observed in the testis tissue of zebrafish exposed to abamectin. (*): Significant differences ($p > 0.05$), (^{a,b,c}) : Dose groups with statistically significant differences according to the Tukey posthoc test results. F.S.T.: Fusion in Seminiferous Tubules, D.S.C.: Decrease in Spermatogonium Count, V.S.T.: Vacuolization in Seminiferous Tubules, D.S.C.C.I.: Disruption of Spermatogenic Cell Cluster Integrity, V.I.T.: Vacuolization in Interstitial Tissue.

use, abamectin is also widely used in veterinary medicine as an antihelmintic against various animal parasites and insects, and it can contribute to water pollution by contaminating water sources in treated areas[10].

This study has provided various findings regarding the potential histopathological effects of abamectin on zebrafish gonads. In the examinations where testis and ovary tissues were assessed separately, it was found that the ovarian tissue exhibited signs of damage in oocytes, the formation of atretic follicles, vacuolization in oocytes, hypertrophy of cortical alveoli, and structural alterations in vitellogenic oocytes. In the testis tissue, widespread atrophy signs were detected, including the blurring of boundaries in seminiferous tubules, degenerated structures in reproductive cells, a decrease in the number of spermatogenic cells, hypertrophy of Leydig cells, fibrosis, an increase in apoptotic and pyknotic cells, and the formation of necrotic areas.

Abamectin, a commonly preferred type of pesticide and a member of macrolide lactone compounds[44], can exhibit multiple toxic effects on various organisms in the ecosystem. Research has shown that abamectin is long-lasting and harmful to different fish species. In a study by Xu et al.[45], ultrastructural examinations revealed no changes in the morphology of *Puntius conchonius* sperm after exposure to 0.14 µM abamectin for 30 min; however, significant damage was observed in the mitochondria located in the middle and surrounding regions of the sperm. Abnormal sperm structures were encountered, showing damage to the head regions and breaks in the middle piece of sperm exposed to 0.42 µM abamectin. In rainbow trout, exposure to abamectin resulted in necrotic spots and vacuolar degeneration in the liver, while degenerative changes were detected in the kidneys and brain[46]. According to El Said (2007)[47], abamectin caused an increase in creatinine levels in *O. niloticus*. This suggests that abamectin affects kidney functions, leading to impaired biochemical catabolism and changes in creatinine levels. In *Oreochromis niloticus-mossambicus* exposed to different doses of abamectin, vacuolization and necrosis in the liver, hyperplasia and edema in the gills, and lesions and necrosis in the intestines were detected[48]. Abamectin causes DNA damage in hepatocytes in *Schizothorax prenanti* and leads to hepatocytic apoptosis in liver cells. Additionally, the type of pesticide has been found to increase the production of reactive oxygen species and caspase activity in correlation with increased dose and duration. It has been reported to cause a decrease in the activities of oxidative stress markers such as superoxide dismutase, glutathione peroxidase, and catalase, along with an increase in malondialdehyde levels[49]. In a study by Kushwaha et al.[6], significant increases

in ALT and AST levels were detected in *Oreochromis mossambicus* exposed to abamectin, indicating its impact on the biochemical values of the liver. Vajargah et al.[50] reported histological analysis of the intestines of *Rutilus caspicus* exposed to abamectin for 96 h revealed disruptions and deformation in intestinal villi and epithelial cells, degeneration in epithelial cells, and the occurrence of necrotic structures in the tissue. While non-high doses of abamectin lead to tissue damage, they also reduce the chances of survival for the organism. Following exposure to abamectin, *Oreochromis niloticus* exhibited elevated levels of ALT and AST, increased MDA levels, decreased total antioxidant capacity, and reduced levels of IgA and IgG[51].

When tested separately, abamectin and difenoconazole were noted to cause toxic effects on zebrafish, with their combined application resulting in more significant toxicity[52]. Histological examinations of the liver of zebrafish exposed to 13, 26, and 53 µg/L of abamectin for 15 days revealed morphological changes in hepatocytes, glycogen accumulation, degeneration in the hepatocyte cytoplasm, and nuclei exhibiting a pyknotic appearance. Changes in body posture, coloration, movement, and opercular activity were observed in fish due to abamectin exposure[39].

Abamectin has also been found to have toxic effects during the early developmental stages of zebrafish. According to Raftery and Volz[53], abamectin causes rapid and reversible hypoactivity in zebrafish embryos, although it does not affect primary motoneuron morphology. It induces rapid neurophysiological effects in early zebrafish embryos. Exposure to 50 µg/L of avermectin has been reported to cause malformations in heart development, changes in heart rate, and reductions in body length. It has also been explained that abamectin alters the expression of heart-related genes, affecting heart development and function, with the heart being the primary target of this chemical[54].

In juvenile zebrafish, exposure to abamectin increased in epithelial cells between the secondary lamellae of the gills, a reduction in respiratory area, expansion at the apex of the secondary lamellae, and separation in the epithelium. It was noted that advanced lesions occur with high exposure levels[55]. According to Sanches et al.[16], aside from low concentrations of abamectin during the early life stages of zebrafish, abamectin application causes a decrease in heart rate. Exposure to abamectin in juvenile zebrafish leads to a reduction in the expression of the cyp1a gene and SOD activity while not affecting glutathione peroxidase and DPPH (1,1-diphenyl-2-picrylhydrazyl) activity[56].

A study by Guru et al.[57] indicated that abamectin exposure was associated with increased levels of reactive oxygen species (ROS) in the ocular region of zebrafish larvae. It was noted that lipid peroxidation products occurred in the ocular region of the larvae due to the induction of apoptosis by abamectin. In the ocular region of zebrafish larvae, increases in O_2 and glutathione were observed. While levels of SOD and CAT decreased, histological analysis revealed damage in the lens region, loss of ganglion cells, and damage to the photoreceptor layer.

In male rats, histopathological examinations of testis tissue following abamectin administration revealed an increase in connective tissue surrounding the seminiferous tubules, areas of hemorrhage in the tissue, and infiltration in blood vessels[58]. In another study investigating abamectin exposure in male rats, damage to testis tissue, disruption of cellular organization, and a decrease in sperm count and motility were observed, indicating an effect on male reproduction. This was attributed to oxidative stress-mediated activation of PARP (poly (ADP-ribose) polymerase). Most experimental studies have shown that pesticides cause such structural damage, impairing reproductive function[59,60], this chemical led to degeneration in some spermatogonium cells, peritubular edema, and reduced spermatogenic cells in testis tissue. Kandil[61] reported that due to abamectin exposure, medullary blood vessels were observed in rat ovarian tissue on the third day, while interstitial stromal cells were detected in the medullary region on the seventh day. Additionally, cellular hypertrophy was noted in the mucosal epithelium of the oviduct. Magdy et al.[62] emphasized that abamectin causes degeneration in spermatogenic cells in male rat testes.

Degenerative changes were detected in the testis tissue of rats following abamectin exposure. The seminiferous tubules exhibited degeneration, atrophy, and vacuolization in the interstitial connective tissue[63].

Studies have observed that different types of pesticides and environmental pollutants produce varying effects on the gonadal structure of zebrafish. Treatment with pyriproxyfen resulted in a decrease in the number of vitellogenic oocytes, an increase in the number of pre-vitellogenic oocytes, and an increase in the number of atretic oocytes due to toxic effects. It was also noted that there was no change in the thickness of the vitelline membrane in vitellogenic oocytes. Still, there was a tendency to induce hypertrophy in the follicular layer. Lipofuscin accumulation was detected in the vitelline membrane[64].

It has been reported that trifloxystrobin, a fungicide, causes a decrease in perinuclear and cortical alveolar oocyte counts in zebrafish ovaries while increasing the percentage of early vitellogenic and late vitellogenic oocytes[65]. Silver nitrate led to a decrease in nutrient granules in mature oocytes and their depletion with increasing doses in zebrafish ovarian tissue. An increase in interstitial connective tissue was also observed. It has been suggested that these chemicals damage the ovaries due to oxidative stress[66]. Similar findings were obtained in our study, and an increase in connective tissue in the interstitial area was observed.

Chlorpyrifos, an organophosphate insecticide increasingly used to control harmful insects from the soil, has been reported to cause structural damage in zebrafish ovaries, damage to the follicular membrane, an increase in the percentage of perinuclear oocytes, and a decrease in the rate of pre-vitellogenic and mature oocytes in the early stages[67]. These findings are also similar to those obtained in our study. Damage to the follicular membrane, a decrease in the number of mature oocytes, and deterioration were also observed in our study.

Developmental abnormalities were observed in the offspring of zebrafish following exposure to Cypermethrin. It was particularly noted that the fertilization stage was disrupted after exposure. This insecticide, like the abamectin used in our study, was found to impair reproduction in zebrafish[68].

Two fungicide types, difenoconazole, have increased the percentages of spermatogonia in the testis tissue of zebrafish. In contrast, tebuconazole and a mixture of these two fungicides resulted in a decrease in the percentages

of spermatocyte cells[69]. In our study, which examined abamectin exposure in male zebrafish exposed to fine particulate matter, we found the presence of degenerated spermatogenic cell clusters, intertubular bleeding, and vacuolization, a decrease in sperm cells, fibrosis, and fusion in some seminiferous tubules[70]. BPA caused histopathological changes and degeneration in the testis tissue of zebrafish, correlating with increasing doses[71]. According to Hu et al.[72], ZPT negatively affects sperm quality and quantity in zebrafish. It causes oxidative damage in the testis tissue, induces apoptosis, and decreases mitochondrial membrane potential. Exposure to benzo[a]pyrene, commonly found in cigarette smoke and exhaust gases, significantly reduced the percentage of spermatozoa in zebrafish while the rate of spermatocytes increased[73].

Chemicals with endocrine-disrupting effects can cause changes in the sexual differentiation or development of species and their reproductive processes[74–77]. Endocrine disruptors such as 17α-ethinylestradiol (EE2) and fadrazole, whether alone or in combination, lead to asynchronous development, interstitial changes, and anomalies such as separation of the basement membrane in the seminiferous tubules in male zebrafish while causing interstitial protein fluid accumulation and an increase in the percentage of atretic oocytes in female individuals[77]. In our study, we also observed the separation of the basement membrane in the seminiferous tubules and increased the number of atretic follicles in female individuals. In the study conducted by Pathirajage and Rajapaksa in 2024[78], like our findings, they observed degeneration in mature oocytes, as well as a reduction in oocyte development and oocyte numbers in the ovaries of zebrafish exposed to Bisphenol A.

Boscalid, a type of fungicide, caused interstitial hemosiderosis (elevated iron levels) and inflammatory cell infiltration in the ovarian tissue of female zebrafish. At the same time, it led to lesions, lysis of Sertoli cells, partial degeneration, and expansion of interstitial tissue in male zebrafish[79]. According to Qiang and Cheng[80], microplastic structures cause a decrease in the thickness of the basement membrane in the testes of male zebrafish but do not cause any change in the oocyte ratios of females. An increase in apoptosis rates was observed in the testes, and elevated ROS levels were noted in the brain, gonads, and liver tissues of both male and female zebrafish. After 21 days of kresoxim-methyl exposure, there was an increase in the percentage of perinuclear and cortical alveolar oocytes in the ovaries of zebrafish, while the percentages of early and late vitellogenic oocytes decreased[81]. Darvishi et al.[82] reported that an increase in diazinon doses led to a decrease in oocyte numbers, while high doses resulted in an increase in atretic follicles and the observation of hyperplastic oocytes. The use of cyprodinil to prevent the infestation of pathogenic fungi led to a decrease in the percentage of primordial follicles, an increase in mature follicles, and a rise in egg production in zebrafish ovaries, along with elevated gonadotropin and testosterone levels[83]. According to Qian et al.[84], pentiopirad caused pathological changes such as follicular atresia, structural abnormalities in the follicles, fusion in cortical vesicles, and structural abnormalities in nutrient granules in the ovarian tissue of female zebrafish. In male zebrafish, it led to expansion in the interstitial area, edema in the interstitium of the testicular tissue, and a decrease in basement membrane thickness. Sharma et al.[85] reported that because of NET application, there was a significant increase in perinucleolar oocytes in the ovaries, while EE2 exposure led to a rise in mature and vitellogenic oocytes but a decrease in perinucleolar oocytes. In the testis tissue, NET exposure was associated with an increase in spermatid and sperm counts and a reduction of spermatocytes. In contrast, EE2 exposure resulted in an increase in spermatocyte counts and a decrease in sperm counts.

Conclusion

This study demonstrates that abamectin, used in various fields, has toxicological effects on the gonads of zebrafish. Based on these results, it can be inferred that it may have adverse effects on reproduction. Applying this chemical has led to histopathological changes by disrupting the morphological characteristics of sex cells, thereby reducing reproductive potential in zebrafish (*Danio rerio*) gonads.

When examining the testicular tissue of zebrafish, findings included indistinct boundaries of seminiferous tubules, loss of structural integrity, degeneration of reproductive cells, hypertrophy of Leydig cells, apoptosis, formation of pyknotic nuclei, fibrosis, and necrotic areas in the interstitial tissue. In the ovaries, there was an increase in the number of atretic oocytes, vacuolization in oocytes, hypertrophy in cortical alveoli, and structural damage in vitellogenic oocytes.

Considering the results obtained, it can be interpreted that abamectin damages the processes of spermatogenesis and oogenesis. In this study, the toxic effects of abamectin on the gonads of zebrafish, a model organism, were identified. To prevent the potential impacts of this substance on living organisms and their release into the environment, its usage should be regulated. Numerous studies have supported the notion that abamectin is toxic to fish, and our findings suggest that this substance also has the potential to induce reproductive toxicity in zebrafish. Furthermore, it is necessary to increase studies on higher organisms and human models to identify potential toxic effects. Therefore, this study highlights the need for further research on this chemical's impact, which could also harm humans.

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

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References

1. Bai, S. H. & Ogbourne, S. Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. *Chemosphere* **154**, 204–214. https://doi.org/10.1016/j.chemosphere.2016.03.113 (2016).

2. Ceylan, S., & Yılmaz, O. Avermektinlerle ilgili son gelişmeler. (1987). http://hdl.handle.net/11452/31937
3. Campbell, W. C. Use of ivermectin in dogs and cats. In: *Ivermectin and abamectin* (pp. 245–259). New York, NY: Springer New York. (1989).
4. El-Saber Batiha, G. et al. Avermectin derivatives, pharmacokinetics, therapeutic and toxic dosages, mechanism of action, and their biological effects. *Pharmaceuticals* **13**(8), 196. https://doi.org/10.3390/ph13080196 (2020).
5. Errami, M. et al. Anodic destruction of abamectin acaricide solution by BDD-anodic oxidation. *Int. J. Electrochem. Sci.* **9**(10), 5467–5478. https://doi.org/10.1016/S1452-3981(23)08182-8 (2014).
6. Kushwaha, S., Anerao, I., Rajput, S., Bhagriya, P. & Roy, H. Evaluation of abamectin induced hepatotoxicity in Oreochromis mossambicus. *Cogent Biol.* **6**(1), 1761277. https://doi.org/10.1080/23312025.2020.1761277 (2020).
7. Novelli, A. et al. Lethal effects of abamectin on the aquatic organisms Daphnia similis Chironomus xanthus and Danio rerio. *Chemosphere* **86**(1), 36–40. https://doi.org/10.1016/j.chemosphere.2011.08.047 (2012).
8. Taşkan, B. Abamektin Pestisitinin Anaerobik Arıtma Sisteminde Mikrobiyal Komunite ve Biyogaz Üretimi Üzerindeki Etkisinin Araştırılması. *J. Institute Sci. Technol.* **11**(3), 1854–1865. https://doi.org/10.21597/jist.813237 (2021).
9. Liang, Y., Dong, B., Pang, N. & Hu, J. ROS generation and DNA damage contribute to abamectin-induced cytotoxicity in mouse macrophage cells. *Chemosphere* **234**, 328–337. https://doi.org/10.1016/j.chemosphere.2019.06.031 (2019).
10. Tišler, T. & Kožuh Eržen, N. Abamectin in the aquatic environment. *Ecotoxicology* **15**, 495–502. https://doi.org/10.1007/s10646-006-0085-1 (2006).
11. Halley, B. A., VandenHeuvel, W. J. & Wislocki, P. G. Environmental effects of the usage of avermectins in livestock. *Veterinary Parasitol.* **48**(1–4), 109–125. https://doi.org/10.1016/0304-4017(93)90149-H (1993).
12. Lewis, K. A., Tzilivakis, J., Warner, D. J. & Green, A. An international database for pesticide risk assessments and management. *Human Ecol. Risk Assess. Int. J.* **22**(4), 1050–1064. https://doi.org/10.1080/10807039.2015.1133242 (2016).
13. Høy, T., Horsberg, T. E. & Nafstad, I. The disposition of ivermectin in Atlantic salmon (Salmo salar). *Pharmacol. Toxicol.* **67**(4), 307–312. https://doi.org/10.1111/j.1600-0773.1990.tb00835.x (1990).
14. Katharios, P., Pavlidis, M. & Iliopoulou-Georgudaki, J. Accumulation of ivermectin in the brain of sea bream, *Sparus aurata* after intraperitoneal administration. *Environ. Toxicol. Pharmacol.* **17**(1), 9–12. https://doi.org/10.1016/j.etap.2004.01.003 (2004).
15. Lasota, J. A., & Dybas, R. A. Avermectins, a novel class of compounds: implications for use in arthropod pest control. https://doi.org/10.1146/annurev.en.36.010191.000515.(1991).
16. Sanches, A. L. M. et al. Lethal and sublethal toxicity of abamectin and difenoconazole (individually and in mixture) to early life stages of zebrafish. *Chemosphere* **210**, 531–538. https://doi.org/10.1016/j.chemosphere.2018.07.027 (2018).
17. Authority, E. F. S. Conclusion regarding the peer review of the pesticide risk assessment of the active substance napropamide. *EFSA J.* https://doi.org/10.2903/j.efsa.2008.140r (2008).
18. Gupta, R. K., Miller, K. P., Babus, J. K. & Flaws, J. A. Methoxychlor inhibits growth and induces atresia of antral follicles through an oxidative stress pathway. *Toxicol. Sci.* **93**(2), 382–389. https://doi.org/10.1093/toxsci/kfl052 (2006).
19. Potashnik, G., Ben-Aderet, N., Israeli, R., Yanai-Inbar, I. & Sober, I. Suppressive effect of 1, 2-dibromo-3-chloropropane on human spermatogenesis. *Fertility Sterility* **30**(4), 444–447. https://doi.org/10.1016/S0015-0282(16)43580-6 (1978).
20. Björvang, R. D. & Damdimopoulou, P. Persistent environmental endocrine-disrupting chemicals in ovarian follicular fluid and in vitro fertilization treatment outcome in women. *Upsala J. Med. Sci.* **125**(2), 85–94. https://doi.org/10.1080/03009734.2020.1727073 (2020).
21. van Duursen, M. B. et al. Safeguarding female reproductive health against endocrine disrupting chemicals—the FREIA project. *Int. J. Molecular Sci.* **21**(9), 3215. https://doi.org/10.3390/ijms21093215 (2020).
22. Aventis, S. Medical Advances And Animal Research The Contribution Of Animal Science To The Medical Revolution: Some Case Histories. RDS: understanding Animal Research in Medicine *and* Coalition for Medical Progress, UK. (2007)
23. Lieschke, G. J. & Currie, P. D. Animal models of human disease: zebrafish swim into view. *Nat. Rev. Genet.* **8**(5), 353–367 (2007).
24. Yipel, M. & Ilhan, A. Zebra Baliği (Danio Rerio): Toksikolojik Çalişmalar Için Uygun Bir Model Organizma. *Veteriner Farmakoloji Ve Toksikoloji Derneği Bülteni* **12**(3), 161–167. https://doi.org/10.38137/vftd.1007548 (2021).
25. Baumann, L. et al. Reversibility of endocrine disruption in zebrafish (Danio rerio) after discontinued exposure to the estrogen 17α-ethinylestradiol. *Toxicol. Appl. Pharmacol.* **278**(3), 230–237. https://doi.org/10.1016/j.taap.2014.04.025 (2014).
26. Dinç, T. 4-T-bütilbenzoik asidin zebra balığı sinir sistemi gelişimi üzerine etkisinin incelenmesi, Investigation of the effect of 4-T-butyl benzoic acid on zebrafish nervous system development. Phd Thesis, Sakarya University. (2023).
27. Di Paola, D. et al. Combined toxicity of xenobiotics Bisphenol A and heavy metals on zebrafish embryos (Danio rerio). *Toxics* **9**(12), 344. https://doi.org/10.3390/toxics9120344 (2021).
28. Schäfers, C. et al. Concentration-and time-dependent effects of the synthetic estrogen, 17α-ethinylestradiol, on reproductive capabilities of the zebrafish, Danio rerio. *J. Toxicol. Environ. Health Part A* **70**(9), 768–779. https://doi.org/10.1080/15287390701236470 (2007).
29. Şişman, T. & Yıldırım, Y. Aflatoksin B1'in Zebra balığının (Danio rerio (Hamilton)) Embriyo ve Larvaları Üzerine Olan Toksik Etkileri. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi* **11**(1), 13–17 (2007).
30. Turhan, D. Ö. Diklofenak Sodyumun Zebra Balığı (Danio rerio) Larvaları Üzerindeki Teratojenik ve Gelişimsel Toksisitesinin Değerlendirilmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi* **26**(1), 183–191. https://doi.org/10.18016/ksutarimdoga.vi.1028753 (2023).
31. Chen, X. et al. Exposure to difenoconazole induces reproductive toxicity in zebrafish by interfering with gamete maturation and reproductive behavior. *Sci. Total Environ.* **838**, 155610. https://doi.org/10.1016/j.scitotenv.2022.155610 (2022).
32. D'Souza, R. C. Assessment of hepatic, ovarian and developmental toxicity in zebrafish due to exposure to low concentrations of di (2-ethylhexyl) phthalate (DEHP). *Journal of Experimental Zoology India*, *26*(2). (2023)
33. Lee, J., Park, J. W., Kim, H. I., Park, C. B. & Cho, S. H. Thyroid-gonadal hormonal interplay in zebrafish exposed to sodium perchlorate: Implications for reproductive health. *Chemosphere* **346**, 140662. https://doi.org/10.1016/j.chemosphere.2023.140662 (2024).
34. Lin, W. et al. Polystyrene microplastics enhance the microcystin-LR-induced gonadal damage and reproductive endocrine disruption in zebrafish. *Sci. Total Environ* **876**, 162664. https://doi.org/10.1016/j.scitotenv.2023.162664 (2023).
35. Mu, X. et al. Environmental level of bisphenol F induced reproductive toxicity toward zebrafish. *Sci. Total Environ.* **806**, 149992. https://doi.org/10.1016/j.scitotenv.2021.149992 (2022).
36. Wang, H. et al. Interference of gut-brain-gonad axis originating from triclocarban exposure to parent zebrafish induces offspring embryonic development abnormality by up-regulation of maternal circSGOL1. *Aquatic Toxicol.* **266**, 106782. https://doi.org/10.1016/j.aquatox.2023.106782 (2024).
37. Yang, R. et al. Insights into the sex-dependent reproductive toxicity of 2-ethylhexyl diphenyl phosphate on zebrafish (Danio rerio). *Environ. Int.* **158**, 106928. https://doi.org/10.1016/j.envint.2021.106928 (2022).
38. Wang, J. Abamectin at environmentally relevant concentrations impairs bone development in zebrafish larvae. *Comparat. Biochem. Physiol. Part C: Toxicol. & Pharmacol.* **287**, 110039 https://doi.org/10.1016/j.cbpc.2024.110039 (2025).
39. Dos Santos, K. P. E. et al. Abamectin promotes behavior changes and liver injury in zebrafish. *Chemosphere* **311**, 136941. https://doi.org/10.1016/j.chemosphere.2022.136941 (2023).
40. Koç, N. D., Aytekin, Y. & Yüce, R. Ovary maturation stages and histological investigation of ovary of the Zebrafish (Danio rerio). *Brazilian Archiv. Biol. Technol.* **51**, 513–522. https://doi.org/10.1590/S1516-89132008000300010 (2008).

41. Akbulut, C. Acute exposure to the neonicotinoid insecticide imidacloprid of zebrafish (*Danio rerio*) Gonads: A histopathological approach. *Ann. Limnol. Int. J. Lim.* **57**, 23 (2021).
42. Akbulut, C. Histopathological and apoptotic examination of zebrafish (*Danio rerio*) gonads exposed to triclosan. 73(4):465–472 (2021b)
43. Agbohessi, P. T. et al. Assessment of the health status of wild fish inhabiting a cotton basin heavily impacted by pesticides in Benin (West Africa). *Sci. Total Environ.* **506**, 567–584 (2015).
44. Huang, Y., Hong, Y., Huang, Z. & He, H. Cytotoxicity induced by abamectin exposure in haemocytes of Chinese mitten crab Eriocheir sinensis. *Environ. Toxicol. Pharmacol.* **77**, 103384. https://doi.org/10.1016/j.etap.2020.103384 (2020).
45. Xu, Y., Zhang, S., Zhang, Y., Hu, J. & Bhattacharya, H. Exposure of rosy barb (*Puntius conchonius*) sperm to abamectin as an in vitro assay of cytotoxicity. *Toxicol. Mechan. Methods* **15**(5), 351–354. https://doi.org/10.1080/15376520500194684 (2005).
46. Jenčič, V., Černe, M., Eržen, N. K., Kobal, S. & Cerkvenik-Flajs, V. Abamectin effects on rainbow trout (Oncorhynchus mykiss). *Ecotoxicology* **15**, 249–257 (2006).
47. El Said, M. M., Evaluation of abamectin toxicity on some biochemical constituents and osmoregulation in freshwater fish Oreochromis niloticus [Tilapia niloticus]. (2007)
48. Thanomsit, C., Ocharoen, Y. & Nanthanawat, P. Behavior, survival rate and histological alterations in Nile tilapia (Oreochromis niloticus-mossambicus) after exposed to abamectin. *Naresuan Univ. J. Sci. Technol. (NUJST)* **24**(3), 65–74 (2016).
49. Hong, Y. et al. Abamectin at environmentally-realistic concentrations cause oxidative stress and genotoxic damage in juvenile fish (*Schizothorax prenanti*). *Aquatic Toxicol.* **225**, 105528. https://doi.org/10.1016/j.aquatox.2020.105528 (2020).
50. Vajargah, M. F., Mohsenpour, R., Yalsuyi, A. M., Galangash, M. M. & Faggio, C. Evaluation of histopathological effect of roach (Rutilus rutilus caspicus) in exposure to sub-lethal concentrations of Abamectin. *Water Air Soil Poll.* **232**(5), 188. https://doi.org/10.1007/s11270-021-05128-w (2021).
51. Mahmoud, H. K., Reda, F. M., Alagawany, M. & Farag, M. R. The stress of abamectin toxicity reduced water quality, growth performance, immunity and antioxidant capacity of Oreochromis niloticus fish: Modulatory role of Simmondsia chinensis extract as a dietary supplement. *Aquaculture* **534**, 736247. https://doi.org/10.1016/j.aquaculture.2020.736247 (2021).
52. Sanches, A. L. M. et al. Single and mixture toxicity of abamectin and difenoconazole to adult zebrafish (Danio rerio). *Chemosphere* **188**, 582–587. https://doi.org/10.1016/j.chemosphere.2017.09.027 (2017).
53. Raftery, T. D. & Volz, D. C. Abamectin induces rapid and reversible hypoactivity within early zebrafish embryos. *Neurotoxicol. Teratol.* **49**, 10–18. https://doi.org/10.1016/j.ntt.2015.02.006 (2015).
54. Du, W., Wang, X., Wang, L., Wang, M. & Liu, C. Avermectin induces cardiac toxicity in early embryonic stage of zebrafish. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **264**, 109529. https://doi.org/10.1016/j.cbpc.2022.109529 (2023).
55. Novelli, A. et al. Impact of runoff water from an experimental agricultural field applied with Vertimec® 18EC (abamectin) on the survival, growth and gill morphology of zebrafish juveniles. *Chemosphere* **144**, 1408–1414. https://doi.org/10.1016/j.chemosphere.2015.10.004 (2016).
56. Hanachi, P., Kazemi, S., Zivary, S., Karbalaei, S. & Ghadami, S. A. The effect of polyethylene terephthalate and abamectin on oxidative damages and expression of vtg and cyp1a genes in juvenile zebrafish. *Environ. Nanotechnol. Monitoring Manage.* **16**, 100565. https://doi.org/10.1016/j.enmm.2021.100565 (2021).
57. Guru, A. et al. Synergetic effects of polyethylene microplastic and abamectin pesticides on the eyes of zebrafish larvae and adults through activation of apoptosis signaling pathways. *Environ. Toxicol. Pharmacol.* **102**, 104215. https://doi.org/10.1016/j.etap.2023.104215 (2023).
58. Elbetieha, A. & Da'as, S. I. Assessment of antifertility activities of abamectin pesticide in male rats. *Ecotoxicol. Environ. Safety* **55**(3), 307–313. https://doi.org/10.1016/S0147-6513(02)00165-3 (2003).
59. Abd-Elhady, H. K. & Abou-Elghar, G. E. Abamectin induced biochemical and histopathological changes in the albino rat, Rattus norvegicus. *J. Plant Protection Res.* https://doi.org/10.2478/jppr-2013-0039 (2013).
60. Celik-Ozenci, C. et al. Effects of abamectin exposure on male fertility in rats: potential role of oxidative stress-mediated poly (ADP-ribose) polymerase (PARP) activation. *Regul. Toxicol. Pharmacol.* **61**(3), 310–317. https://doi.org/10.1016/j.yrtph.2011.09.001 (2011).
61. Kandil, R. A. Sexual Hormones And Pathological Changes In Female Albino Rats Rattus Norvegicustreated With Abamectin Biocide. *Egyptian Journal Of Agricultural Research*, 93. (2015).
62. Magdy, B. W., Mohamed, F. E., Amin, A. S. & Rana, S. S. Ameliorative effect of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney and testis of male albino rats. *J. Basic Appl. Zool.* **77**, 69–82. https://doi.org/10.1016/j.jobaz.2016.10.002 (2016).
63. Hassan, H. F. & Meligi, N. M. Effects of sublethal abamectin exposure on some hormonal profiles and testicular histopathology in male albino rats and the possible ameliorative role of Eruca sativa. *Environ. Sci. Poll. Res.* **24**, 24690–24697 (2017).
64. de Oliveira, V. S. et al. In vivo exposure to pyriproxyfen causes ovarian oxidative stress and inhibits follicle maturation in zebrafish. *J. Appl. Toxicol.* **43**(6), 799–807. https://doi.org/10.1002/jat.4425 (2023).
65. Chen, L. et al. Trifloxystrobin induced developmental toxicity by disturbing the ABC transporters, carbohydrate and lipid metabolism in adult zebrafish. *Chemosphere* **349**, 140747. https://doi.org/10.1016/j.chemosphere.2023.140747 (2024).
66. Ramchandani, D. M., Modi, C. M., Patel, P. M., Patel, H. B., Patel, U. D., Paida, B. V. & Patel, H. R. Silver nitrate damages the ovary of adult zebrafish: Evaluation of oxidative stress, expression of oxidative stress-related genes and histological changes in ovary. https://doi.org/10.21203/rs.3.rs-3254429/v1.(2023)
67. Ma, J. et al. Environmental impacts of chlorpyrifos: Transgenerational toxic effects on aquatic organisms cannot be ignored. *Sci. Total Environ.* **905**, 167311. https://doi.org/10.1016/j.scitotenv.2023.16731 (2023).
68. Han, S., Liu, X., Liu, Y. & Lu, J. Parental exposure to Cypermethrin causes intergenerational toxicity in zebrafish offspring. *Sci. Total Environ.* **935**, 173456 (2024).
69. Jiang, J. et al. Histology and multi-omic profiling reveal the mixture toxicity of tebuconazole and difenoconazole in adult zebrafish. *Sci. Total Environ.* **795**, 148777. https://doi.org/10.1016/j.scitotenv.2021.148777 (2021).
70. Manjunatha, B., Seo, E., Bangyappagari, D. & Lee, S. J. Histopathological and ultrastructural alterations reveal the toxicity of particulate matter (PM2. 5) in adult zebrafish. *J. Hazardous Mater. Adv.* **7**, 100135. https://doi.org/10.1016/j.hazadv.2022.100135 (2022).
71. Özkan-Kotiloğlu, S., Arslan, P., Akca, G. & Günal, A. Ç. Are BPA-free plastics safe for aquatic life?-Fluorene-9-bisphenol induced thyroid-disrupting effects and histopathological alterations in adult zebrafish (Danio rerio). *Comparative Biochem. Physiol. Part C Toxicol. Pharmacol.* **260**, 109419. https://doi.org/10.1016/j.cbpc.2022.109419 (2022).
72. Hu, J. et al. Toxic effects and potential mechanisms of zinc pyrithione (ZPT) exposure on sperm and testicular injury in zebrafish. *J. Hazardous Mater.* **461**, 132575. https://doi.org/10.1016/j.jhazmat.2023.132575 (2024).
73. Xu, K. et al. Benzo (a) pyrene exposure in early life suppresses spermatogenesis in adult male zebrafish and association with the methylation of germ cell-specific genes. *Aquatic Toxicol.* **258**, 106504. https://doi.org/10.1016/j.aquatox.2023.106504 (2023).
74. Fenske, M., Maack, G., Schäfers, C. & Segner, H. An environmentally relevant concentration of estrogen induces arrest of male gonad development in zebrafish, Danio rerio. *Environ. Toxicol. Chem. Int. J.* **24**(5), 1088–1098. https://doi.org/10.1897/04-096R1.1 (2005).
75. Hill, R. L. Jr. & Janz, D. M. Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. Effects on sex ratio and breeding success. *Aquatic Toxicol.* **63**(4), 417–429. https://doi.org/10.1016/S0166-445X(02)00207-2 (2003).

76. Kinnberg, K., Holbech, H., Petersen, G. I. & Bjerregaard, P. Effects of the fungicide prochloraz on the sexual development of zebrafish (Danio rerio). *Comparative Biochem. Physiol. Part C Toxicol. Pharmacol.* **145**(2), 165–170. https://doi.org/10.1016/j.cbpc.2006.11.002 (2007).
77. Luzio, A., Monteiro, S. M., Rocha, E., Fontaínhas-Fernandes, A. A. & Coimbra, A. M. Development and recovery of histopathological alterations in the gonads of zebrafish (Danio rerio) after single and combined exposure to endocrine disruptors (17α-ethinylestradiol and fadrozole). *Aquatic Toxicol.* **175**, 90–105. https://doi.org/10.1016/j.aquatox.2016.03.014 (2016).
78. Pathirajage, K. S. & Rajapaksa, G. Long-term exposure to environmentally relevant Bisphenol-A levels affects growth, swimming, condition factor, sex ratio and histology of juvenile zebrafish. *Sci. Rep.* **14**(1), 24503 (2024).
79. Qian, L. et al. Exposure to boscalid induces reproductive toxicity of zebrafish by gender-specific alterations in steroidogenesis. *Environ. Sci. Technol.* **54**(22), 14275–14287. https://doi.org/10.1021/acs.est.0c02871 (2020).
80. Qiang, L. & Cheng, J. Exposure to polystyrene microplastics impairs gonads of zebrafish (Danio rerio). *Chemosphere* **263**, 128161. https://doi.org/10.1016/j.chemosphere.2020.128161 (2021).
81. Fang, N. et al. Histology and metabonomics reveal the toxic effects of kresoxim-methyl on adult zebrafish. *Chemosphere* **309**, 136739. https://doi.org/10.1016/j.chemosphere.2022.136739 (2022).
82. Darvishi, M. et al. Sublethal doses of diazinon affected reproductive, immune, and oxidative status in female zebrafish (Danio rerio). *Aquaculture Rep.* **22**, 100944. https://doi.org/10.1016/j.aqrep.2021.100944 (2022).
83. Tang, C., Zhu, Y., Yang, C., He, C. & Zuo, Z. Reproductive toxicity of long-term exposure to environmental relevant concentrations of cyprodinil in female zebrafish (Danio rerio). *Sci. Total Environ.* **846**, 157504. https://doi.org/10.1016/j.scitotenv.2022.157504 (2022).
84. Qian, L. et al. Assessment of reproductive toxicity in adult zebrafish (Danio rerio) following sublethal exposure to penthiopyrad. *Ecotoxicol. Environ. Safety* **268**, 115721. https://doi.org/10.1016/j.ecoenv.2023.115721 (2023).
85. Sharma, A., Kumari, P. & Sharma, I. Experimental exploration of estrogenic effects of norethindrone and 17α-ethinylestradiol on zebrafish (Danio rerio) gonads. *Comparative Biochem. Physiol. Part C Toxicol. Pharmacol.* **275**, 109782. https://doi.org/10.1016/j.cbpc.2023.109782 (2024).

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Author contributions

All authors contributed to the manuscript's conception and design. The authors declare no competing interests.

Declarations

Competing interests

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