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RESEARCH PAPER

Genotoxic Effect, Oxidative Stress and Cell Death due to Metronidazole Application in Gills and Liver Tissues of Rainbow Trout (Oncorhynchus mykiss)

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Abstract
In this study, the purpose was to investigate the histopathological, genotoxic effect, oxidative stress and cell death due to Metronidazole (MTZ), which is a 5-nitroimidazole compound, used widely for the treatment of anaerobic organism infections in fish and humans on gill and liver tissues of *Oncorhynchus mykiss*. Trout fishes were exposed to 5, 10, and 20 mg/L of MTZ in the aquariums for 2, 4 and 8 days. Staining technics namely H&E, NOS immunohistochemistry, and TUNEL were performed to determine histopathological changes, oxidative damage and apoptosis. Additionally, smear preparations were also prepared from gill blood for genotoxic evaluations. The organ damage started in the 2nd day with 5 mg/L MTZ application and effects increased per duration and dose-dependent manner. It was observed that the gills had the primary and secondary lamellae lengths, with formation of clavate lamellae, fusion in secondary lamellae, separation of epithelium and aneurysm. Regional necrosis, vacuolization of hepatocytes, pycnotic nucleus, enlarged sinusoids were also determined in the liver. NOS immunoreactivity increased with the inducible immunoreactivity (iNOS) that was more prominent when compared to the endothelial immunoreactivity (eNOS). Apoptotic immunoreactivity was higher in the 10 mg 8th day experimental group at liver and gills, and was lower 20 mg 8th day experimental group. When the gills and liver compared with each other, in all doses, immunoreactivity was lower in gills, compared with liver. Genotoxic examinations showed that both number of **micro nucleated** erythrocytes and nuclei abnormalities were higher in MTZ-treated groups.

Keywords: Metronidazole, fish, oxidative damage, cell death, genotoxicity

INTRODUCTION

Metronidazole (MTZ) is a nitroimidazole that is employed in many countries for treatment of protozoan infections like *Trichomanas vaginalis, Entamoeba histolytica*,*Giardia lamblia* (Hernández Ceruelos et al., 2019),anaerobic bacteria (Zhao et al., 2018) and treatment of many parasitic diseases (Forouzesh et al., 2019). However, it was also determined that it has many serious side effects such as neurological disorders linked to the central nervous system (Goolsby et al., 2018, Altunışık and Eke Kurt, 2021), endocarditis (Lee, 2016), tumor formation and mutagenic disorders (Adil et al., 2018, Hernández Ceruelos et al., 2019). IARC, (1987) argued that MTZ could be carcinogenic in humans and in animals. It was also found that it causes single stranded breaks in human and animal DNA (EMA, 1997).

Histological investigation of fish tissues are a useful tool for detecting environmental changes

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and its effect in water (Fernandez et al., 2011, de Lima Cardoso et al., 2018). Therefore, toxicological studies that made in this field are very important for saving biological life and protecting the nature. Based on this thought, changes in gill and liver tissues, which are vital organ for fishes, has been observed with both histopathologic and genotoxic methods. Fish are sensitive to acute and chronic environmental changes and can create a classic stress response. When the immune system cell population is activated in acute stress conditions, they can release cytokines that can cause reactive oxygen metabolites, such as chemokines, growth factors, arachidonic acid and Nitric Oxide (NO). NO is an inorganic free radical that is released by various cells, especially by the epithelial cells. It is converted into nitrate and nitrite shortly after it is synthesized (Oliveira-Paula et al., 2019). It was reported in previous studies that increased nitrate rates have negative impacts, such as the occurrent infections, various physiological stresses, weakened immune system, tissue damage, as well as the inability to transport enough oxygen in the blood, resulted with the development of kidney failure on aquatic life (Shimura et al. 2004, Camargo et al. 2005). NO can be transformed into highly effective radicals in many physiological mechanisms by acting as a free radical and by reacting with reactive oxygen types, such as superoxide (Ghimire et al., 2017, Gantner et al., 2020). Increased NO causes cytotoxicity and DNA damage, affects enzymes containing iron sulfur, disrupts mitochondrial respiration and triggers cell death such as apoptosis or necrosis, because of the cells glycolytic capacity (Zhang, 2018, Poderoso et al., 2019) by Reactive Oxygen Species (ROS) (Lepetsos and Papavassiliou, 2016). Intense and chronic oxidative stress in the organism exceeding the detoxification limits and endurance capacity of trabecular network cells, causes destruction of cytoskeletal structures, reduces adhesiveness and finally, disrupts cell integrity (Thurmond et al., 2015, Wilson and González-Billault, 2015). MTZ's genotoxic activity has been examined through *in vitro* and *in vivo* tests. From which, it was reported that MTZ has a mutagenic effect on bacteria and could even cause mutations together with DNA recombination in fungi (De Méo et al., 1992). Fish are organisms commonly used in genotoxic studies, especially in micronucleus test studies (Van der Oost et al., 2003, Çavaş and Ergene-Gözükara, 2005). For this reason, our study also intended to investigate the genotoxic effects of MTZ by using the erythrocyte micronucleus test in *Oncorhynchus mykiss*.

In our previous studies, histopathological changes on intestinal connective tissue caused by MTZ exposure, especially at the epithelial tissue, decreased immunostaining on matrix proteins like laminin and collagen IV (Gürcü et al., 2016), and organ damage that caused by oxidative stress and apoptotic cell death has been observed (Gürcü et al., 2017). In this study, investigation of oxidative stress with dose dependent manner in gill and liver tissues in fish that exposed to MTZ, and cell death with damage dependent mechanisms from the stress data, shown above, has been purposed.

MATERIALS AND METHODS

Rainbow trout (*Oncorhynchus mykiss*) from Bozdoğan/Aydın (total n = 90) farm were brought to the laboratory and were placed in previously prepared aquariums. They were treated optimal conditions. The fish were divided into 3 groups as positive (cyclophosphamide, CP), negative (drug-free) control, and application group. The application group fish were divided into 3 groups again, and each group was mixed into MTZ aquarium water with 5-10-20 mg/L. The gill and liver tissue samples (n = 10) were taken after 2 days (Group I), 4 days (Group II), and 8 days (Group III) from the fish of each group. The samples of all tissues were fixed with 10% buffered neutral formalin for 24-48 hours. After the dehydration with alcohol, it was clarified with xylol and embedded in paraffin. Routine Hematoxylin-Eosin (H&E) staining, NOS immunohistochemistry and TUNEL labeling were performed by taking 5µm sections of the tissues. The sections were examined in Olympus light microscope, and were then evaluated by taking pictures with Olympus (DP 74) camera in different sizes (Mete et al., 2021).

Histopathological scoring for assessment of gill and liver produced by modification of

previous protocols (Rodrigues t al. 2017). Modification was made by scoring of the pathological alteration. Each alteration was assessed using a "score value" ranging from 1 to 6 (mild to severe occurrence) depending on the degree and extent of the alteration. To evaluate the oxidative stress, immunohistochemistry analysis of gills and liver tissues was performed using endothelial and inducible nitric-oxide synthase reagents (eNOS and iNOS), the technical specifications of these reagents were eNOS (SCBT, sc-654) and iNOS (SCBT, sc-651) (Aldoghachi et al., 2016).

The immunohistochemical procedure was performed three times, and the immunohistochemical data was given as H-score. The evaluation of stained samples was done gradually; weak (+), moderate (++) and strong (+++) respectively. Besides, the immunopositive cells were counted for each staining degree. The H-score formula was used as: H-Score = Pi (intensity of staining + 1). Pi means the percentage of stained cells for each intensity. All the slides were examined by the same observer who was blind to the tissue sections between control and experimental groups (Debaugnies et al., 2016, Mete et al., 2021)

The TUNEL (Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling) specified as (Millipore, S7101). Method was used to detect the apoptotic cells in situ (Mete et al., 2021). Fish blood was smeared on clean microscopic slides for genotoxicity assessment. These blood smear slides were air dried and then fixed in absolute methanol for 20 minutes. Slides were stained for 20 minutes in 5% Giemsa and washed in distilled water and left to dry. The samples were examined in Olympus BX 51 microscope, and photographs were taken with Olympus E-330 digital camera. Approximately 1.000 cells were counted from each group.

The obtained data were tested with One-Way Analysis of Variance (ANOVA) to evaluate possible changes statistically with a significant p value ($p<0.05$). Statistical analyses were made with GraphPad Prism (Version 9).

RESULTS AND DISCUSSION

The morphology of the control group was normal in gill tissue, primary and secondary lamellae, and the secondary lamellae extending toward the surfaces of the gills. Secondary lamellae consisted of epithelial cells that were parallel to the surface, capillary endothelium and cells of pillar cells between these cells (Figure 1). Application group gill tissues had shortening and

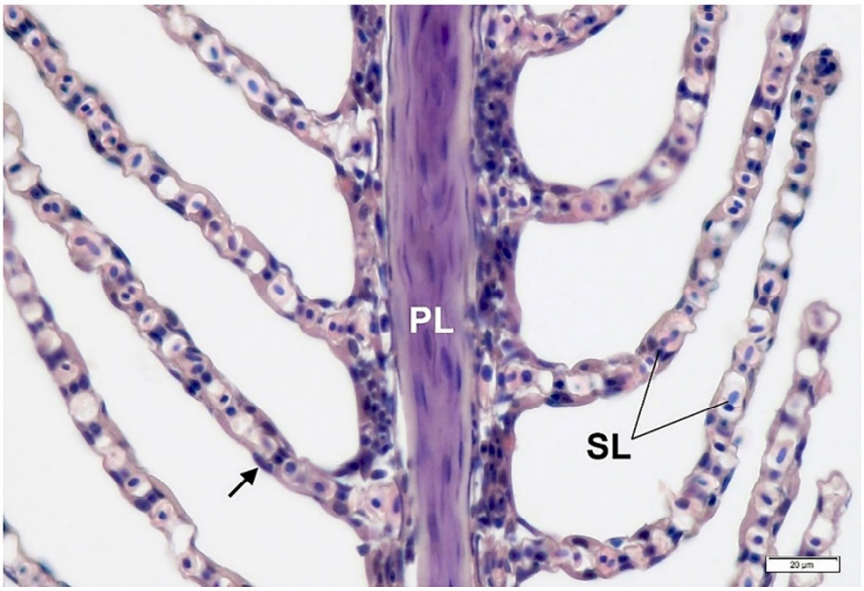

Fig. 1. Control group gill tissue sample. PL: Primary lamellae, SL: Secondary lamellae →: Epithelial cells. **Staining;** H-E, **Magnification;** 20 µm.

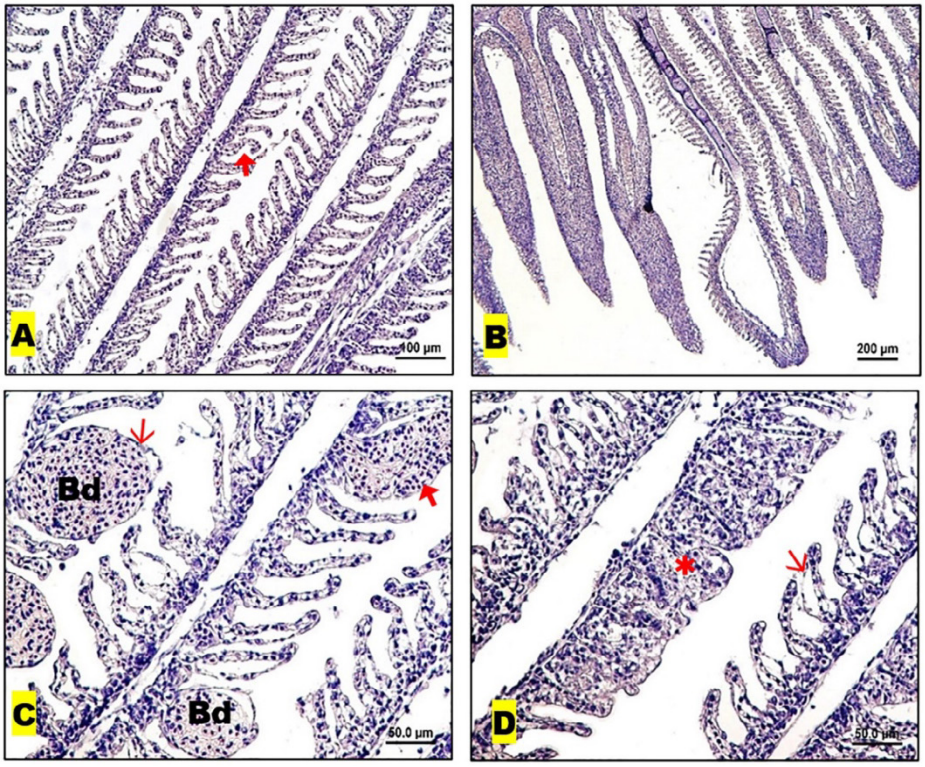

Fig. 2. Changes observed in gill tissues in application group. **A:** 5 mg MTZ, **B:** 10 mg MTZ, **C-D:** 20 mg MTZ. Separation in epithelium (→), blood cell accumulation (Bd), clavate lamellae formation (➔), hyperplasia (✱). **Staining;** H-E, **Magnification;** A: 100 µm, B: 200 µm, C-D: 50 µm.

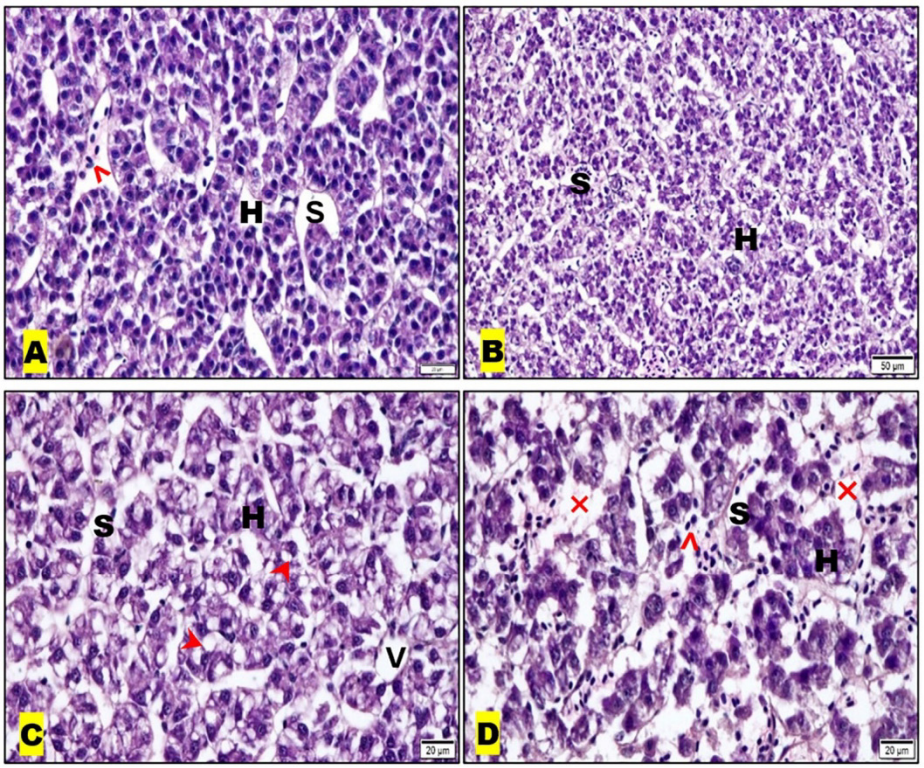

Fig. 3. A; Control group liver tissue. **B, C, D**; Changes observed in liver tissues in application group, H; Hepatocytes, vacuolization (⌂), necrosis (x), erythrocytes (^), sinusoid (S). (**B:** 5 mg MTZ, **C:** 10 mg MTZ, **D;** 20 mg MTZ). **Staining;** H-E, **Magnification;** A, C, D: 20 µm, B:50 µm.

shredding in primary (Figure 2A) and in secondary lamellae epithelial lengths depending on dose (Figure 2A, C). Separation of secondary lamellae epithelium, (Figure 2C, D) hyperplasia (Figure 2D) and clavate lamellae formation (Figure 2A, C), and accumulation of blood cells caused by expansion of capillary in secondary lamellae (Figure 2C).

The control group showed normal liver tissue hepatocytes in the form of polygonal cells with a single round nucleus or double nuclei. Capillary blood vessels and erythrocytes were observed between the cell cords formed by hepatocytes side by side (Figure 3A). The application group was filled with enlarged capillaries and erythrocyte in the cell cords depending on dose (Figure 3B, D). It is also worth noting that vacuolization and cellular integrity were impaired in hepatocytes (Figure 3C). The total histopathology score of these results are given in Figure 4.

Considered as oxidative stress indicators, eNOS and iNOS immunohistochemistry staining reagents showed basal level in control group. eNOS staining between secondary epithelium and undifferentiated basal cells in gill tissues was more pronounced than in iNOS (Figure 5A, B). Depending on the dose in the gill tissues of the application group, iNOS staining intensity was higher compared to that of eNOS (Figure 6A, B). Generally, staining using eNOS (Figure 7A, B, C, D) and iNOS (Figure 8A, B, C, D) were less in the liver tissues (Figure 9A) compared to those of gill tissues (Figure 9B). Staining of iNOS was more pronounced than that of eNOS. Moreover, iNOS staining was increased depending on the dose.

As a marker for apoptosis, although the TUNEL-positive cells were not detected in the

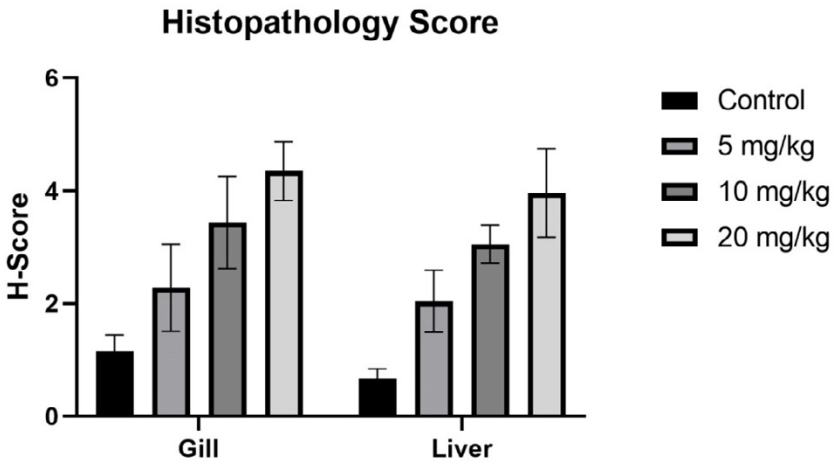

Fig. 4. Histopathology score graph of gill and liver tissues.

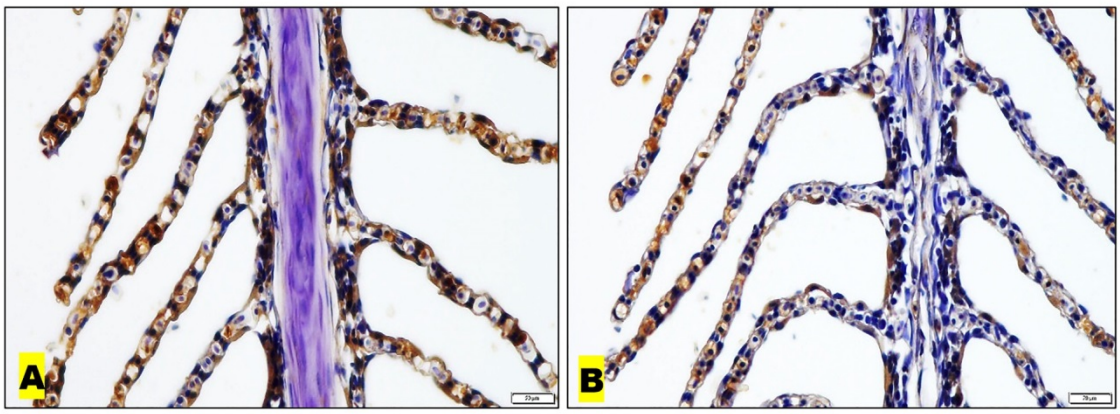

Fig. 5. Control group gill tissue eNOS **(A)**, iNOS **(B)** immunohistochemistry staining. Bar: A, B: 20 μm.

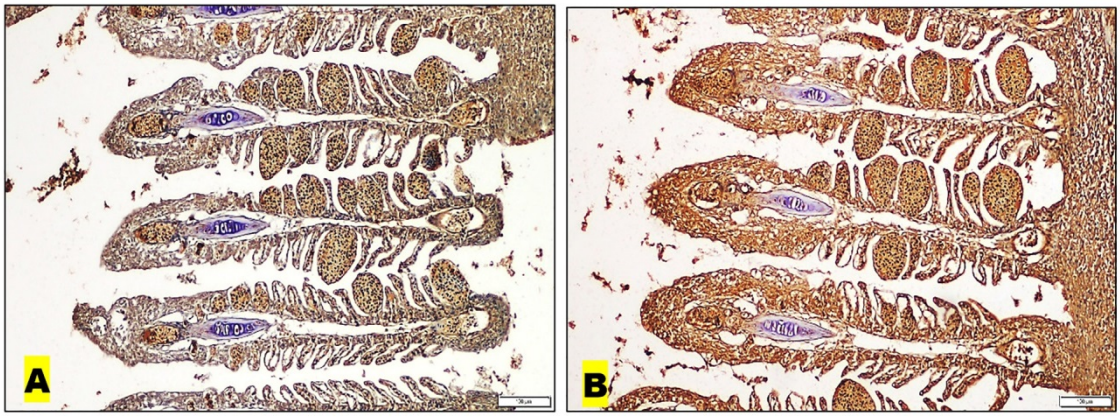

Fig. 6. Application group gill tissue eNOS **(A)**, iNOS **(B)** immunohistochemistry staining. **Magnification;** A, B: 100 µm.

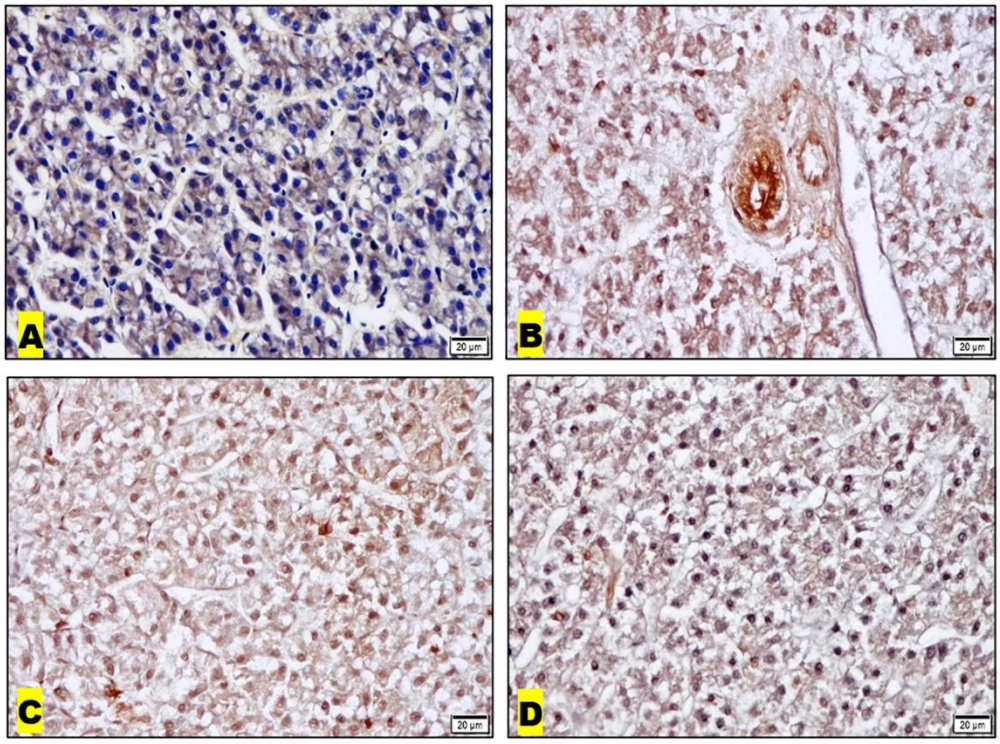

Fig. 7. Control (A) and application group (**B:** 5 mg, **C:** 10 mg, **D:** 20 mg MTZ) eNOS staining of liver tissue. **Magnification;** A, B, C, D: 20 µm.

control group's gill (Figure 10) and liver tissues (Figure 11), MTZ caused apoptosis and necrosis in other MTZ application groups. The positive cells decreased as the dose increases. Several TUNEL-positive cells were found in the liver tissues. Total apoptotic indices of both tissues are given at Figure 12.

In the present study, various nucleus abnormalities like binucleus, blebbed nucleus, notched nucleus, and micronucleus anomalies were found in the 3 different groups of *O. mykiss* that were treated with different doses of MTZ. Micronucleus and nucleus abnormality types, rates, intragroup and intergroup statistical data of the groups are given in Table 1; photos of nucleus abnormalities are given in Figure 13.

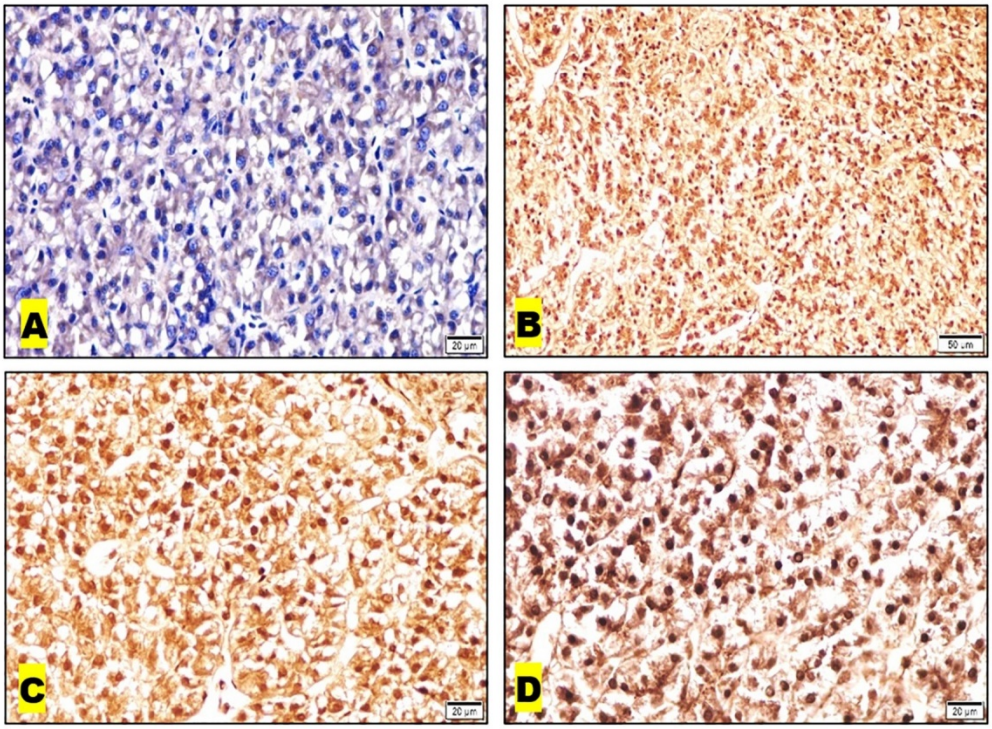

Fig. 8. Control **(A)** and application group (**B:** 5 mg, **C:** 10 mg, **D:** 20 mg MTZ) liver tissue iNOS staining. **Magnification;** A, C, D: 20 µm, B: 50 µm.

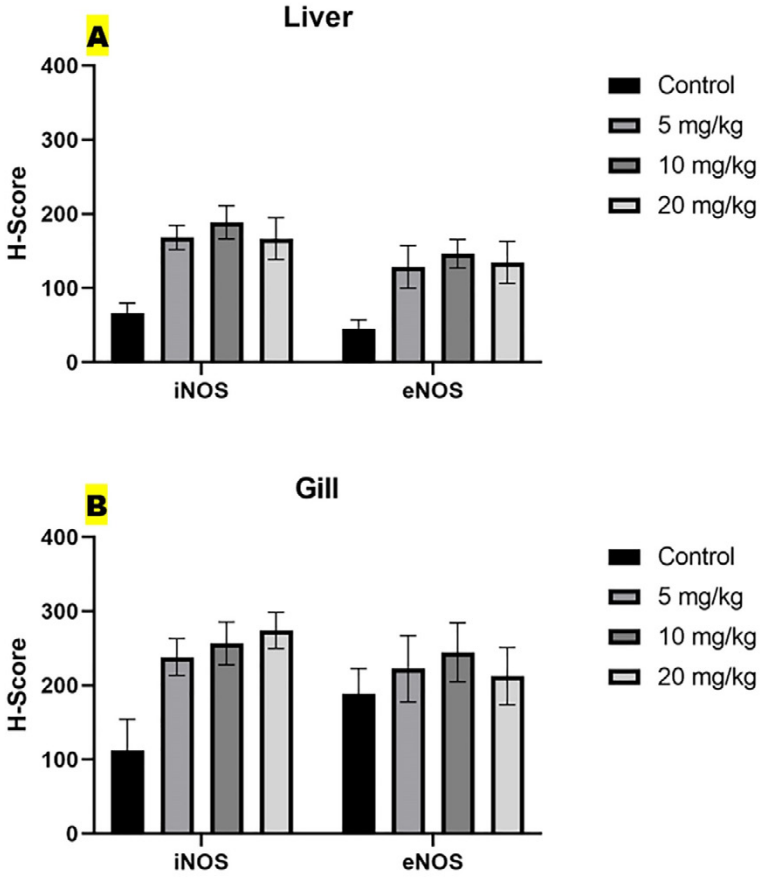

Fig. 9. Hscore graphs of gill and liver immunohistochemical staining (**A:** liver, **B:** gill).

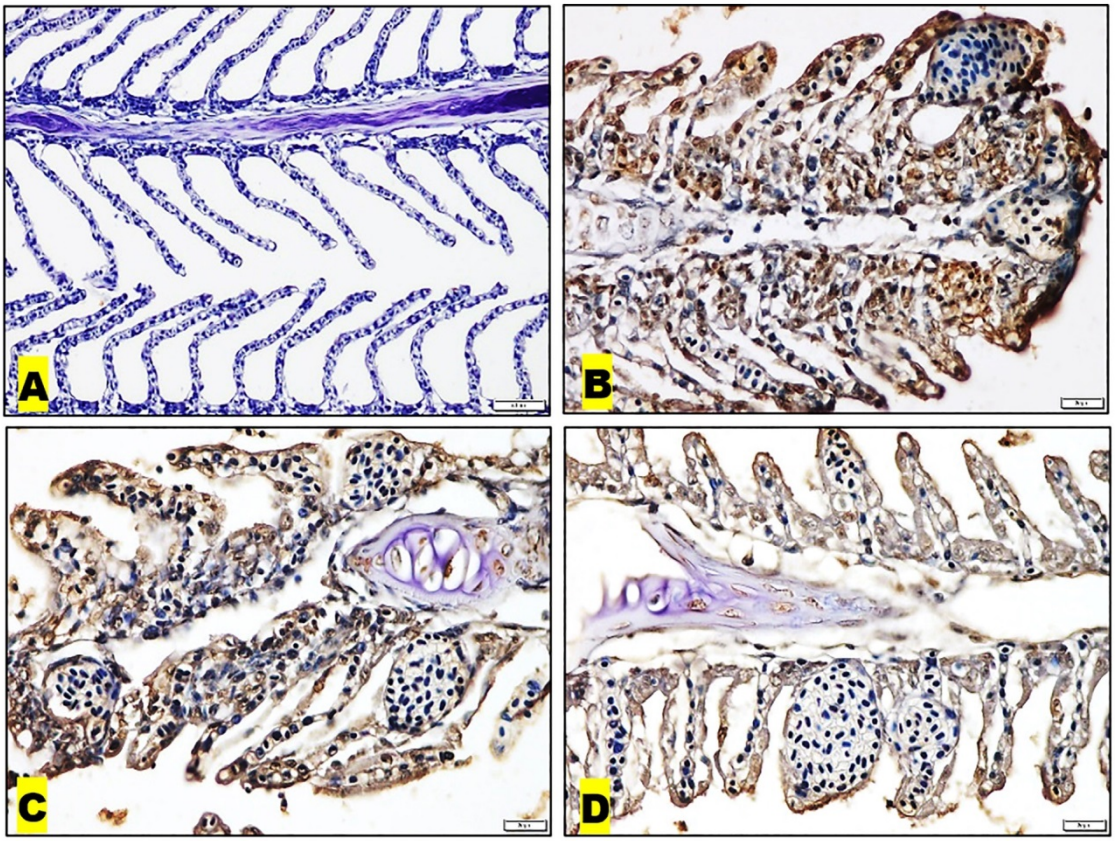

Fig. 10. Control **(A)** and application group (**B:** 5 mg, **C:** 10 mg, **D:** 20 mg MTZ) gill tissue TUNEL staining. **Magnification; A:** 50 µm, **B**, **C**, **D:** 20 µm,

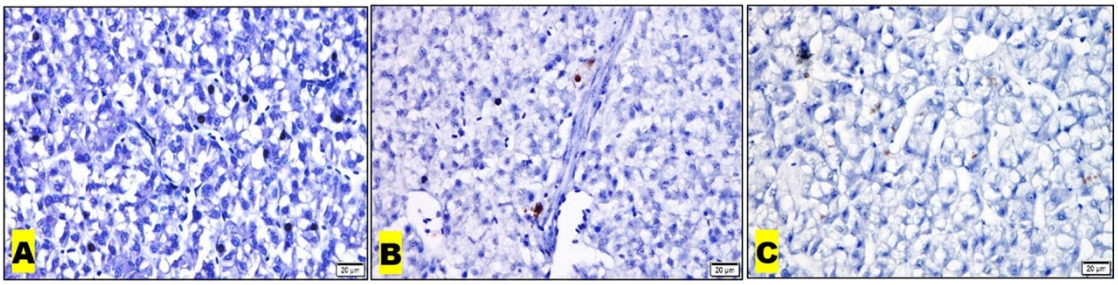

Fig. 11. Control **(A)** and application group (**B:** 5 mg, **C:** 20 mg MTZ) liver tissue TUNEL staining. **Magnification; A, B, C:** 20 µm

As seen in Table 1, the rate of binucleus abnormality for 5 mg was higher in the 1st Group than 10 and 20 mg. In the 2nd Group, the rate for binucleus was statistically significant in 20 mg application compared to the control, 5 and 10 mg/L groups. For the 3rd Group, the binucleus rate in 5 mg/L was not significant compared to the control group but it was significant for 10 and 20 mg/L. Considering the blebbed nucleus trait, the difference between the control and positive control was significant compared to the 1st group for 5 and 10 mg/L. While the differences with the last two groups (Group 2 and 3) were statistically significant concerning the notched nucleus, the differences between the application groups the control group were not significant.

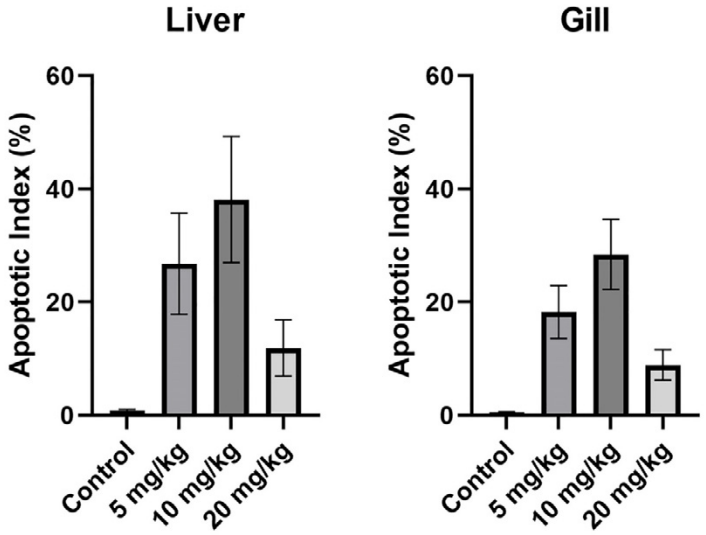

Fig. 12. Apoptotic index percentages of gill and liver tissues.

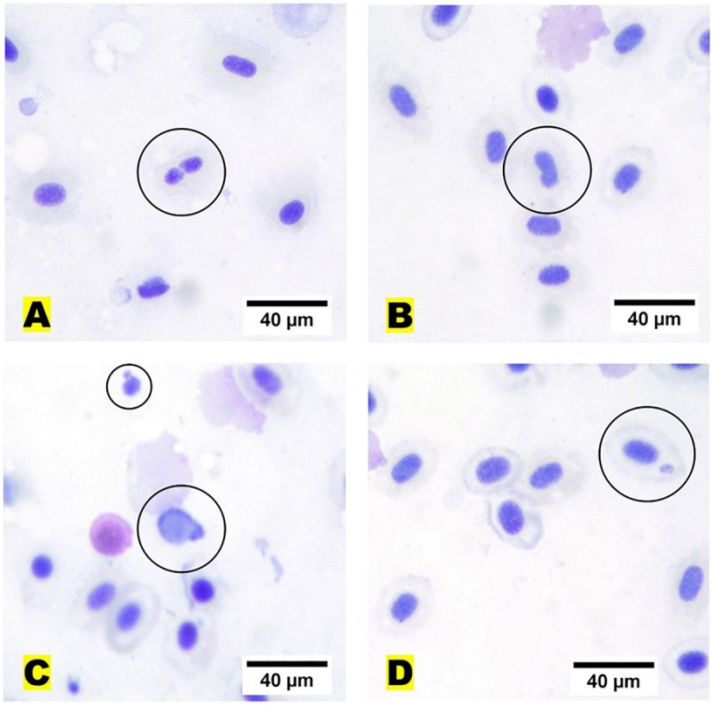

Fig. 13. Nucleus abnormalities caused by the treatment with MTZ. A. Binucleus (BN), B. Notched nucleus (NT), C. Blebbed nucleus (BL), D. Micronucleus (MN). **Magnification; 40X**

Micronucleus formation after 10 mg/L application for Group 2 was significantly higher than all the other groups.

Antibiotics that are used for animal and human health blend into the nature either directly or indirectly and enter terrestrial and aquatic media (Välitalo et al., 2017). It is already known that these active substances that are used for treatment have the potential to cause harm and affect the organisms that living in these environments negatively. It was reported that this might be a worrying outcome for humans. (Langaoen et al., 2018).

MTZ, which is a 5-nitroimidazole compound that used in protozoal and anaerobic bacterial

Table 1. The types and rates of nucleus abnormalities detected as a result of the treatment with different MTZ doses. **BN.** Binucleus; **BL.** Blebbed; **NT.** Notch; **MN.** Micronucleus.

	Doses	**Counted cell**	**BN***	**BL***	**NT***	**MN***
	Control	1006	0.2ac	3.4a	0.4a	---a
	Positive control	1035	---a	5.8b	1abf	0.4ac
1. Group	5 mg	1005	3.4b	1.2c	2.2bcf	0.4bc
	10 mg	1007	0.8ae	1.4ce	3cd	0.6bc
	20 mg	1006	0.4ac	2.6ae	1.6ac	0.4ac
2. Group	5 mg	1010	0.8ae	16d	0.4a	0.2ac
	10 mg	1007	1ae	18.6f	4d	1.4d
	20 mg	1012	10d	8.8g	0.8af	0.6bc
3. Group	5 mg	1015	0.4ac	19f	2.2bcf	0.4ac
	10 mg	1025	1.6e	7.2hj	1.2af	0.2ac
	20 mg	1029	1.2ce	7.8gh	2bcf	0.2ac

*: The means indicated by the same letter are not significant at 0.05.

infections, has negative effects on beneficial bacteria in the biological filter, and causes the accumulation of nitrate in water (Turgut, 2017). It is already well-known that it has negative effects in increasing nitrate content in water and in promoting the infection of living beings (Shimura et al., 2004, Camargo et al., 2005). Also, toxic substances negatively affect anatomy and the physiology by causing acute stress in living organisms. In this respect, the toxic effect of MTZ, which is used as an antibiotic, was examined in this present study, and its association with oxidative stress was highlighted**.**

In fish, gills are the first tissue to face water, and therefore, are a good model for investigating the effects of toxic substances on living tissues (Rodrigues et al., 2017). Gills are the widest contact surfaces of fish with the aquatic environment, and therefore toxic substances can cause excessive damage to its cells. Toxicants taken with the gill epithelia reach other organs through the bloodstream (Aldoghachi et al., 2016).

One of the first changes on fish gills that are exposed to toxic substances is the separation in the epithelial tissues. The purpose is to prevent toxicant entry by increasing the distance between the external environment and the blood flow (Rodrigues et al., 2019). Other morphological changes observed were the formation of edema in the form of excessive hypertrophy and hyperplasia of lamellae epithelial cells and chloride cells. The purpose of these changes in tissues is to minimize the exposed surfaces of the gill and therefore respiratory surface to reduce toxicant entry (Aldoghachi et al., 2016, Rodrigues et al., 2017, Rodrigues et al., 2019).

It is suggested that a circulatory disorder that is caused by the accumulation of large amounts of blood in secondary lamellae, which is also defined as aneurism, is caused by the complete collapse of the Pillar Cell System, which forms the gill epithelium to protect against both mechanical wear and infection (Hassaninezhad et al., 2014). Rodrigues et al., (2019)

reported that xenobiotics causes this pathological condition, and also detected aneurism in the gills compared to control group of fish (*O. mykiss*), however, this condition could not be associated with the presence of the tested compound and then could be considered as a natural formation. Similarly, to our findings, our study showed that there was increase in aneurisms depending on time and dose. The necrosis in the gills disappears completely from the soft tissue covering the gill filaments and causes the exposition of the cartilage part, which results in a decrease in the performance of respiratory task of the gill. For this reason, the overall health of the fish is affected, which can even lead to the death (Strzyżewska et al., 2016)

The MTZ-related effects that has been found in the liver of *O. mykiss* are in the form of hepatocellular necrosis, enlargement of sinusoids and accumulation of many red blood cells. It shows similarity to the findings in liver samples of the fish exposed to Oxytetracycline and Erythromycin (Rodrigues et al., 2017, Rodrigues et al., 2019). In lower doses, MTZ ensures tissue health through its immunomodulation effects causes immediate damages when used in high doses. Various degrees of histopathological changes cause cytoplasmic lysis, pycnotic nucleus and necrosis, also, it could lead to a high metabolic activity in hepatocytes in response to the increase in contaminants. Another study conducted on Oxytetracycline showed areas of bleeding in rat liver and kidney tissues and concluded that this might be caused by increased oxidative stress as a result of the accumulation of free radicals (Gnanasoundari and Pari, 2006).

Oxidative stress-producing processes are similar in aquatic organisms and mammals. Many xenobiotics might induce the production of reactive oxygen species through various biochemical mechanisms such as membrane-bound electron transport (e.g. mitochondrial, microsomal electron transport), and accumulation of reduced intermediary substances, redox cycle, photosensitization, facilitation of Fenton reaction, resulting in the accumulation of hydroxyl radicals, ineffective antioxidant enzymes and depletion of free radical cleaners (Slaninova et al., 2009). In this context, it is also reported the negative effect of free radicals' activity over the detoxification pathways of reactive oxygen metabolites, pairs. When cells encounter these, the cell response after their accumulation is death by either apoptotic or necrotic mechanisms depending on duration and dose (Oset-Gasque et al., 2004). In our study, the relationship of damage to gill and liver tissues exposed to MTZ with these mechanisms was examined, and it was seen that our findings were consistent with those reported by Ronza et al., (2011). In the study conducted by Rodrigues et al., (2017) on *O. mykiss*, it was reported that oxytetracycline application can similarly cause oxidative damage and genotoxic effect, which means that the histological changes in the gill and liver observed in our study may be caused by oxidative stress. Recent studies also showed that many xenobiotics, including drugs like metronidazole, have toxic effects, and the mutagenic potential of MTZ may be associated with a decrease in the nitro group and the formation of reactive oxygen species (Han et al., 2013, Onopiuk et al., 2018).

Respecting the same doses, the occurrence of liver necrosis is controversial, in some previous studies, necrosis was observed (Wojtacka et al. 2011), while it was not in some other research (Rodrigues et al. 2017). In our study, the large number of necrotic areas, both in the liver and gill, is more important than apoptosis areas. Besides, in this context, it was reported by Nakonechna et al. (2018) that the xenobiotics might cause large amounts of hepatocyte loss through necrosis and apoptosis, and the activation of the cell death pathway could cause inflammation and formation of many diseases. A similar mechanism can be considered to occur in the gill and liver tissues. In this study, histopathological examinations revealed the damage of MTZ application on the gill and liver tissues, showing that the gills were the most affected amongst the other tissues.

At the end of our genotoxic examinations, it was found that the nucleus anomalies with erythrocyte count and micronucleus were higher in the groups treated with MTZ compared to the control group. Similar studies were conducted by different researchers by using different test materials, and it was shown that this substance had a genotoxic effect (Lanzky and Halting-

Sørensen, 1997).

In the literature search, there is not much novel studies has been found, considering the genotoxic effect of MTZ. In the given literatures below, there is mentions for contradictory results considering the toxicity of MTZ. The research done with MTZ in humans by Mitelman et al., (1976) reported that MTZ causes increased rate of chromosomal anomaly. In different research published later, it has been reported that there is no genotoxic effect has been found (Mitelman et al., 1976, Lambert et al., 1979). Research done by Elizondo et al., (1996) showed that at the test subjects that took therapeutic doses of MTZ are showed to induce chromatic and isochromatic breaks in their lymphocytes. Mudry et al., (1994) has reported that the MTZ shows clastogenic effect and causes significant increase on chromosomal anomalies, micronucleus formation, and abnormal metaphase rates. Horváthová et al., (1998) has been reported that clastogenic activity of MTZ is stemmed from unpaired strand breaks in chromosomal level while cell cycle continues.

Different drugs that applied to fishes also gave similar results in other researches. Wide spectrum antibiotic Oxytetracycline (OTC) was applied to Cyprinus carpio in 80 mg/L dose in four different timelines, and when three different tissues was investigated, compared to control group, anomalies like micronucleus, nuclear anomalies, swollen cells and cells with vacuoles has been observed. It has been determined that most damage is occurred in gill cells. It has been observed that 24 and 72 hours of OTC exposure can increase polychromatic eritrocyte (PCE) count, and 96 hours of exposure can lower the count significantly. Increased count of PCE might be stemmed from demand for blood cells because of respiratory stress in fishes, and decrease in PCE at 96 hours of exposure might be stemmed from excessive damage to the genetic material, chromosomal break and cell death that caused by OTC (Sharma et al., 2019a). Another research done in C. carpio with paracetamol exposure shows micronucleus (MN), binucleus (BN), blebbed nucleus (BL), fragmented DNA, lobbed nucleus, notched nucleus like nuclear anomalies and cytoplasmic anomalies like cytoplasmic vacuole and karyolysis in cells. Nuclear and cytoplasmic anomalies in blood cells that caused by paracetamol can be the result of the tendency of producing reactive oxygen intermediates (ROI) and reactive nitrogen intermediates while paracetamol metabolizes. Possibility of reaction of these reactive metabolites with protein and nucleic acids, and termination of DNA replication and DNA repair has been suggested by (Sharma et al., 2019b). In 10 and 20 mg/L doses, it might have been caused DNA damage with increased doses, and eventually, increased BL cell count. Cells that have BL formed as a result of excessive DNA amplification and protruding of this excess DNA to the nuclear membrane (Shimizu et al., 1998, Ergene et al., 2007).

There are scientific studies that shows the toxic effect of MTZ in fish and some aquatic species (Khalil et al., 2007, Talapatra et al., 2010). From the research by Mitrowska et al., (2015) on *O. mykiss* with MTZ and from the research by Gadaj et al., (2015) on *Penaeus monodon* with 3-nitroimidazole was found that this drug's metabolites was present in animal tissues. Because of MTZ and its metabolites have mutagenic, cancerogenic and toxic properties, it has been thought that continuous input of MTZ in environment might cause long-term important effects on ecosystem stability. Because of this, developed countries are banned MTZ on use for animal husbandry and fishery purposes. At the analyses done from fish and shrimp tissue showed that there is nitroimidazole compounds inside and their metabolites are present in their meat. Based on this information, we can tell that fish and other food sources that are contaminated with nitroimidazole compounds can cause various histopathological and genotoxic effects on humans. In the present study, various nucleus abnormalities i.e. binuclei, blebbed nucleus, notched nucleus, and micronucleus were all detected. These findings are similar to the results of other researchers. (Talapatra et al., 2010). The increase in the number of micronucleus is important in that it provides us with the proportion of damage to the genetic material.

Shimizu et al., (1998) are reported that amplified DNA is located around specific nuclear

zones and eliminated with nuclear budding to form micronucleus. It has been suggested that the MTZ is reduced biologically to a hydroxylated metabolite by cytochrome P450 enzyme system (Çavaş and Ergene-Gözükara, 2005). The presence of -OH group in this metabolite can increase activity of macromolecules like DNA, RNA and proteins. This MTZ metabolite is known as more potent mutagen and can damage the DNA more than MTZ itself (Dobiáš et al., 1994).

CONCLUSION

As a conclusion, it was found that MTZ can cause damage directly to fishes and indirectly to humans. The outcomes of this study emphasize once again that caution should be taken in the use of this drug.

Further holistic studies confronting these qualities with the effect of environmental pollutions are required and would be serve humanity with great contribution.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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