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INVESTIGATION OF THE HISTOPATHOLOGICAL EFFECTS OF 5-FLUOROURACIL AND CISPLATIN EXPOSURE ON THE MANTLE TISSUE OF *Melanopsis praemorsa* LINNAEUS, 1758 (GASTROPODA: PROSOBRANCHIA)

Şehriban TOKAT[1,*], Birgül OTLUDİL[2]

[1]*Department of Biology,* Institute of Science*, Dicle University, 21280 Diyarbakır, Turkey*
sehribantkt.99@gmail.com
[2]*Department of Biology, Faculty of Science, Dicle University, 21280 Diyarbakır, Turkey*
birgulotludil@gmail.com

ABSTRACT

In recent years, the accumulation of pharmaceutical pollutants in aquatic environments and their ecological toxicity have emerged as critical concerns in environmental sciences and ecotoxicology. Among these, antineoplastic agents are of particular concern due to their potent biological activity and persistence in the environment. Chemotherapeutic drugs such as 5-Fluorouracil and Cisplatin, which are widely used in cancer treatment, are not efficiently removed by conventional wastewater treatment processes. Consequently, they can enter surface waters and pose potential risks to non-target aquatic organisms. Hence, the application of reliable biomarkers is essential for accurate environmental toxicity assessments.

In the present study, the freshwater gastropod *Melanopsis praemorsa* was exposed to environmentally relevant single and combined concentrations (4.56 µg/L^{-1}, 7.91 µg/L^{-1}, 124 µg/L^{-1}, and 250 µg/L^{-1}) of 5-Fluorouracil and Cisplatin to evaluate their histopathological effects. The exposures were conducted for 7, 14, and 21 days. At the end of each exposure period, mantle tissues were dissected and processed for histological analysis under a light microscope. Histopathological alterations observed in the mantle tissue due to 5-Fluorouracil and Cisplatin exposure included muscle fiber atrophy, degeneration of cilia, increased lipid vacuolization, epithelial desquamation, and necrosis. These alterations are indicative of tissue-level toxicity and provide compelling evidence of the sublethal effects of these antineoplastic agents on aquatic invertebrates. The findings also support the potential use of *Melanopsis praemorsa* as a sensitive bioindicator species in aquatic environmental monitoring. This study contributes significantly to the understanding of the toxicological impact of pharmaceutical contaminants in freshwater ecosystems and highlights the need to incorporate antineoplastic compounds into environmental risk assessment frameworks.
Keywords: *Melanopsis praemorsa*, 5-Fluorouracil, Cisplatin, histopathology, antineoplastic agent.

1. INTRODUCTION

In recent years, the substantial increase in the consumption of pharmaceutical compounds has led to their uncontrolled release into the environment. Notably, a significant portion of these chemicals reaching the environment through hospital waste, domestic use, and industrial discharges consists of antineoplastic (chemotherapeutic) drugs [1,2]. Due to their high biological activity, antineoplastic agents are indispensable for human health in cancer treatment. These drugs are often excreted from the body either unmetabolized or in biologically active forms, and subsequently enter surface and groundwater systems without being effectively removed by conventional wastewater treatment plants [3,4,5]. The inability of these facilities to adequately eliminate such compounds results in their presence at ecologically relevant concentrations in surface, ground, and even drinking water, posing various toxic risks to non-target organisms [3,6,7]. Studies conducted in the early 1970s in the United States revealed the presence of numerous pharmaceutical substances in wastewater. Advances in analytical methodologies over time have facilitated the detection of pharmaceuticals at trace levels in surface water, wastewater, groundwater, and drinking water [8].

5-Fluorouracil and Cisplatin are potent cytotoxic antineoplastic drugs widely used in medical treatments due to their mechanisms that inhibit cell division. These compounds have been shown to exert genotoxic, cytotoxic, and histopathological effects in aquatic organisms, posing a serious threat to ecosystem health [9,10,11,12]. Aquatic invertebrates such as benthic and slow-moving gastropods are particularly at risk of prolonged exposure to such persistent pollutants and are considered important model organisms in ecotoxicological studies due to their bioaccumulation potential [13].

Among freshwater gastropods, *Melanopsis praemorsa* is considered a suitable model organism for such studies. Its sensitivity to environmental stressors and broad geographical distribution make it a promising bioindicator species for monitoring the biological impacts of pollutants [14,15,16].

Histopathological analysis is a widely used sensitive biomarker technique to determine cellular-level alterations in the tissues of organisms as a direct consequence of exposure to environmental pollutants [17,18]. Histological examinations serve as valuable tools for assessing the extent of pollution, particularly for sublethal and chronic effects, by providing evidence of pollutant exposure [19,20,21].

The aim of this study is to investigate the histopathological changes in the mantle tissue of the freshwater snail *M. praemorsa* following exposure to 5-Fluorouracil and Cisplatin, and to evaluate the potential of this species as a biomarker in the monitoring of pharmaceutical pollution.

2. MATERIALS AND METHODS

M. praemorsa specimens (average shell length: 8–10 mm; average weight: 380–400 mg) were collected from a freshwater source located in the Bismil district of Diyarbakır and transported to the laboratory. The snails were placed in a glass aquarium (40 × 35 × 40 cm) within a climate-controlled room and acclimatized to laboratory conditions for 20 days. Lighting was maintained on a 16-hour light / 8-hour dark photoperiod. Throughout the acclimation and experimental phases, the laboratory temperature was held constant at 22 ± 1°C. During the acclimation period, the snails were fed once daily with lettuce leaves (*Lactuca sativa*), and aquarium waste was removed once daily [19,22,23].

Following acclimation, the snails were randomly assigned to eight groups, consisting of one control and seven experimental groups. Each group included 30 individuals, which were placed in glass jars containing 2 L of aerated, dechlorinated water. For the experimental groups, 5-Fluorouracil and Cisplatin were applied either individually or in combination at concentrations reflecting their environmental presence in surface and wastewater. The environmentally detected concentration of 5-Fluorouracil in wastewater was 124 µg/L^{-1}, while its predicted environmental concentration (PEC) in surface water was 7.91 ng/L^{-1} [10,24,25]. For Cisplatin, the wastewater concentration was 250 µg/L^{-1} and the surface water PEC was 4.36 ng/L^{-1} [10,26,27].

The experimental groups were organized as follows:

1. **Group 1 – Control (n=30):** Snails were treated with physiological saline equivalent in volume to the drug solutions used in treatment groups.
2. **Group 2 – Positive control (n=30):** Snails were exposed to 8 mg/L^{-1} Bisphenol A (BPA), known for its genotoxic effects, in 50 mL of distilled water [28,29].
3. **Group 3 – 5-Fluorouracil (surface water level) (n=30):** 5-Fluorouracil was applied at 7.91 ng/L^{-1} in 50 mL of physiological saline.
4. **Group 4 – 5-Fluorouracil (wastewater level) (n=30):** 5-Fluorouracil was applied at 124 µg/L^{-1} in 50 mL of physiological saline.
5. **Group 5 – Cisplatin (surface water level) (n=30):** Cisplatin was applied at 4.36 ng/L^{-1} in 50 mL of physiological saline.
6. **Group 6 – Cisplatin (wastewater level) (n=30):** Cisplatin was applied at 250 µg/L^{-1} in 50 mL of physiological saline.
7. **Group 7 – Combined 5-Fluorouracil + Cisplatin (surface water levels) (n=30):** 5-Fluorouracil and Cisplatin were applied in combination at 7.91 ng/L^{-1} + 4.36 ng/L^{-1} in 50 mL of physiological saline.
8. **Group 8 – Combined 5-Fluorouracil + Cisplatin (wastewater levels) (n=30):** 5-Fluorouracil and Cisplatin were applied in combination at 124 µg/L^{-1} + 250 µg/L^{-1} in 50 mL of physiological saline.

On days 7, 14, and 21 of the experiment, five snails were randomly selected from each treatment and control group. The snails were dissected and mantle tissues were collected and fixed in 10% formalin solution. Fixed tissues were rinsed under tap water for 24 hours. Samples were then dehydrated through a graded ethanol series (30%, 50%, 70%, 80%, 90%, 96%, and 100%), cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were obtained using a microtome (LEICA), stained with Hematoxylin and Eosin (H&E), and examined under a light microscope (Olympus BX51). Histopathological alterations were photographed using a camera (Olympus DP7) attached to the microscope.

3. RESULTS

In the control group, the mantle was lined with a single-layered ciliated columnar epithelium. The muscle tissue consisted of densely packed muscle fibers, while the connective tissue contained lipid vacuoles and mucus cells [30,31,19]. "As shown in Figure 1A, no histopathological changes were observed in the mantle tissues in the control group".

“As shown in Figure 1B, structural changes such as an increase in lipid vacuoles, epithelial shedding and cilia degeneration were observed in the mantle tissue on the 7th and 14th days after exposure to Bisphenol A at a concentration of 8 mg L⁻¹, and these changes reached statistically significant levels on the 21st day”.

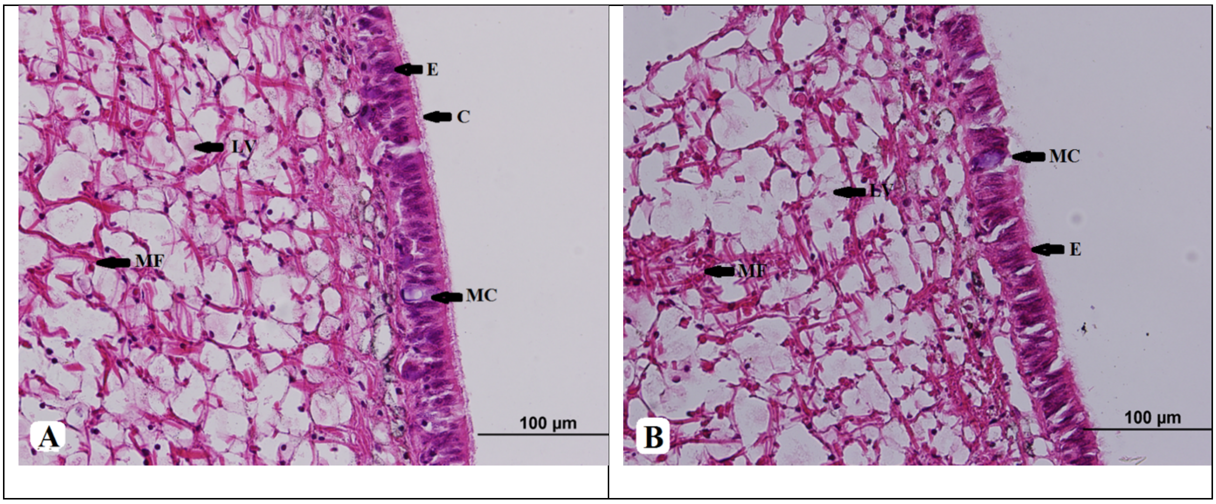
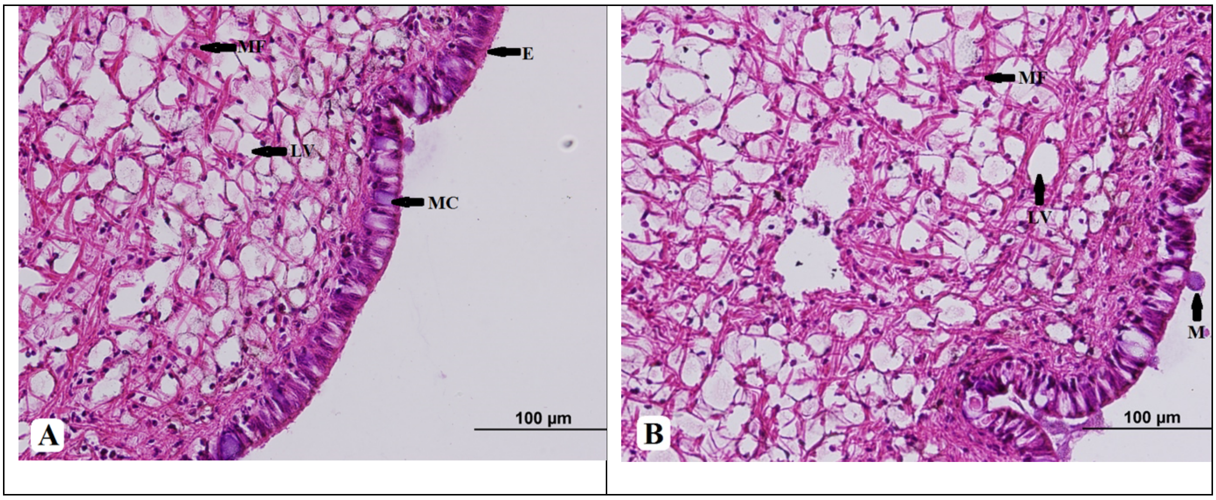


Figure 1. Histological structure of *M. praemorsa* mantle tissue in control on day 21 (A), snails exposed to 8 mg L^{-1} Bisfenol A on day 21 (B). (E epithelium, C cilia, MC mucus cell, LV lipid vacuole, MF, muscle fiber). H&E.

“As shown in Figures 2A, 3A, exposure to 5-Fluorouracil at a concentration of 7.91 ng/L^{-1} caused mild structural changes in the mantle tissue and an increase in mucus cells on days 7 and 14. By day 21, these changes reached a significant level”. Histopathological findings included epithelial desquamation, mild atrophy of muscle fibers, and an increase in both the number of lipid vacuoles and mucus secretion.

“As shown in Figures 2B, 3B, in the group exposed to 124 µg/L^{-1} 5-Fluorouracil, moderate structural changes in the mantle tissue and an increase in mucus cells and their secretion were observed on days 7 and 14”. By day 21, progressive and significant histopathological damage became apparent, including marked epithelial desquamation, complete degeneration of cilia, and a significant accumulation of lipid vacuoles.



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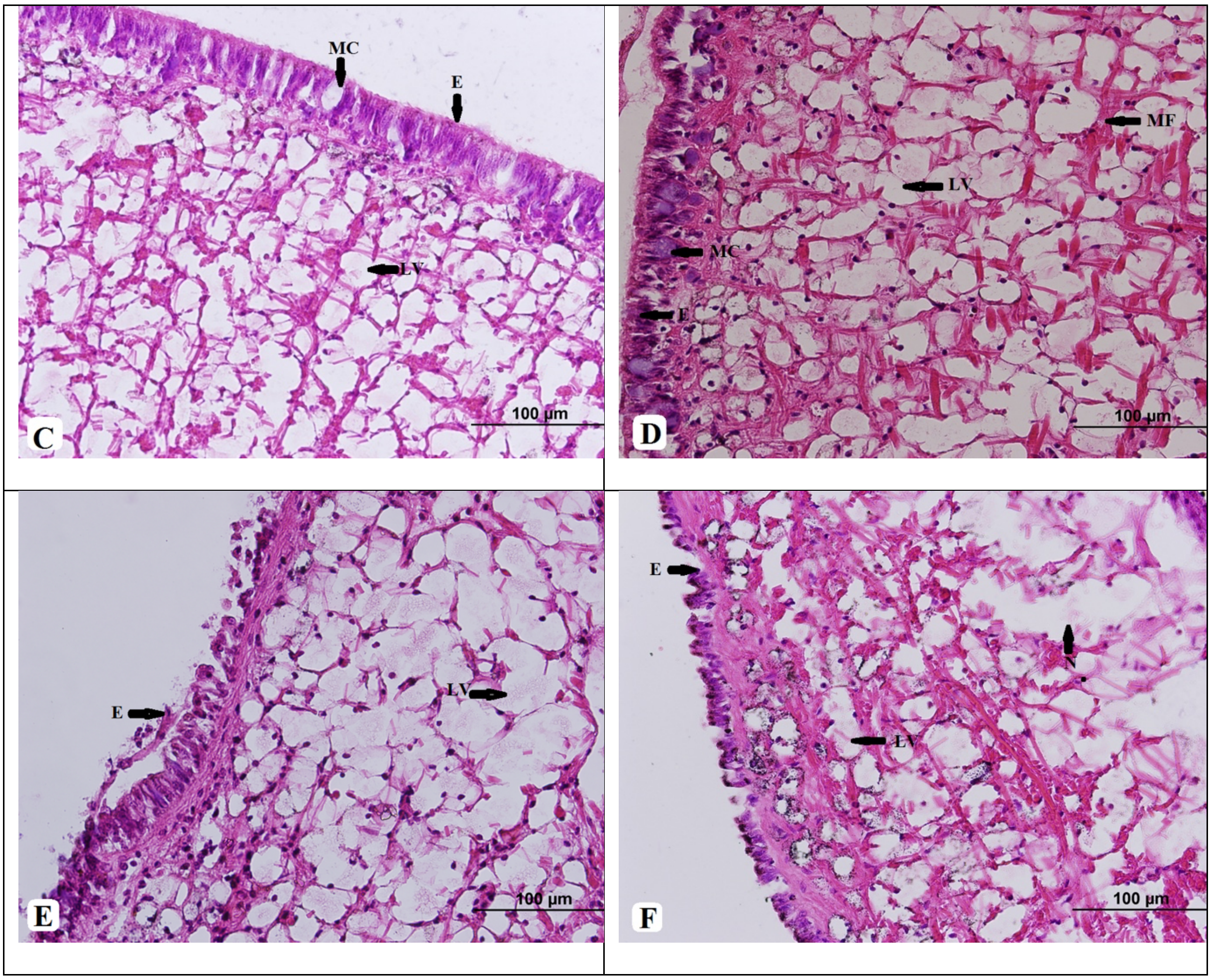
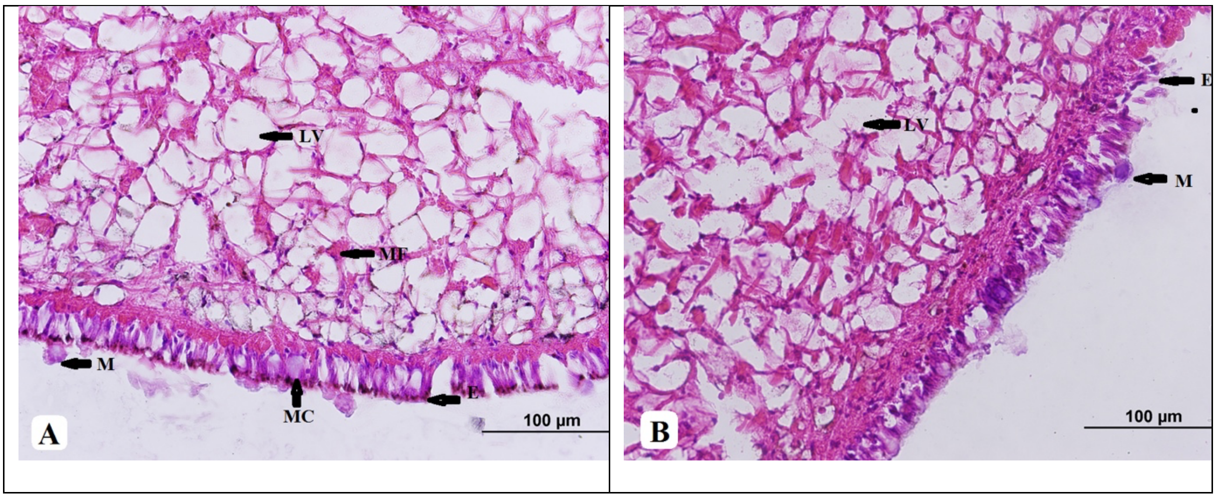


Figure 2. Histological structure of *M. praemorsa* mantle tissue in snails exposed to 7.91 ng/L^{-1} 5-Fluorouracil on day 7 (A), snails exposed to 124 µg/L^{-1} 5-Fluorouracil on day 7 (B), snails exposed to 4.56 ng/L^{-1} Cisplatin on day 7 (C), snails exposed to 250 µg/L^{-1} Cisplatin on day 7 (D), snails exposed to 7.91 ng/L^{-1} + 4.56 ng/L^{-1} 5-Fluorouracil + Cisplatin on day 7 (E), snails exposed to 124/µg L^{-1} + 250 µg/L^{-1} 5-Fluorouracil + Cisplatin on day 7 (F). (E epithelium, MF, muscle fiber, C cilia, MC mucus cell, LV lipid vacuole, M mucus, N necrosis). H&E.

"As shown in Figures 2C, 3C, exposure to 4.56 ng/L^{-1} Cisplatin caused mild structural changes in the mantle tissue on days 7 and 14, and these changes became evident on day 21". Histopathological changes included epithelial desquamation, moderate atrophy of muscle fibers, and an increase in the number of lipid vacuoles.



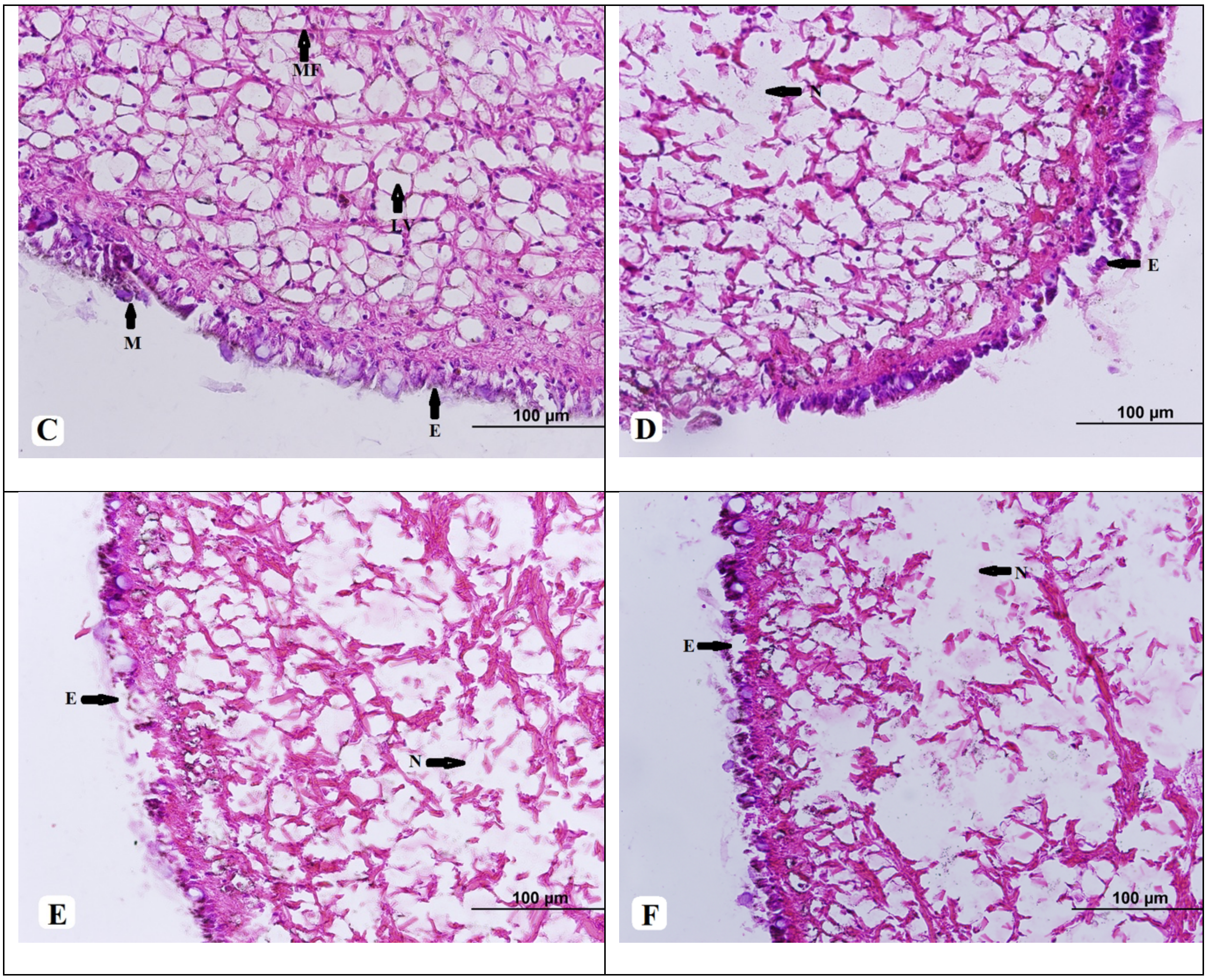


Figure 3. Histological structure of *M. praemorsa* mantle tissue in snails exposed to 7.91 ng/L^{-1} 5-Fluorouracil on day 21 (A), snails exposed to 124 µg/L^{-1} 5-Fluorouracil on day 21 (B), snails exposed to 4.56 ng/L^{-1} Cisplatin on day 21 (C), snails exposed to 250 µg/L^{-1} Cisplatin on day 21 (D), snails exposed to 7.91 ng/L^{-1} + 4.56 ng/L^{-1} 5-Fluorouracil + Cisplatin on day 21 (E), snails exposed to 124 µg/L^{-1} + 250 µg/L^{-1} 5-Fluorouracil + Cisplatin on day 21 (F). (E epithelium, MF, muscle fiber, C cilia, MC mucus cell, LV lipid vacuole, M,mucus, N necrosis). H&E.

"As shown in Figures 2D, 3D, after exposure to 250 µg/L^{-1} Cisplatin, a significant increase in mucous cells was observed on days 7 and 14. On day 21, severe epithelial desquamation, advanced ciliary degeneration, marked muscle fiber atrophy, widespread lipid vacuole accumulation, and obvious necrosis were seen in the mantle tissue".

"As shown in Figures 2E, 3E, combined exposure to 5-Fluorouracil (7.91 ng/L^{-1}) and Cisplatin (4.56 ng/L^{-1}) resulted in significant increases in epithelial desquamation and lipid vacuole accumulation in the mantle tissue on days 7 and 14. On day 21, severe epithelial desquamation, increased lipid vacuolization, and necrosis were observed".

"As shown in Figures 2F, 3F, moderate degeneration and necrotic areas were detected in the mantle tissue on days 7 and 14 in the group exposed to the combination of 5-Fluorouracil (124 µg/L^{-1}) and Cisplatin (250 µg/L^{-1}). By day 21, severe epithelial desquamation, advanced muscle fiber atrophy, and widespread necrosis were among the most prominent histopathological findings".

4. DISCUSSION

In this study, the histopathological effects of 5-Fluorouracil and Cisplatin exposure on the mantle tissue of *Melanopsis praemorsa* were comprehensively evaluated. The findings demonstrated that both agents induced structural alterations in the mantle tissue, which intensified in a dose- and time-dependent manner. In particular, epithelial desquamation, hyperplasia of mucus cells, and an increase in lipid vacuoles were observed. These toxic effects were prominent in the tissues and were found to be consistent with previous studies on heavy metals and pesticides conducted in different gastropod species [19,22,32,33]. These results clearly suggest that the chemotherapeutic agents in question can cause tissue damage in aquatic invertebrates.

The observed increase in mucus cells may be interpreted as a protective response by the organism against toxic agents. Enhanced mucus production may serve to strengthen the epithelial barrier and reduce direct contact with harmful substances [34]. However, such cellular defense mechanisms appeared to be insufficient under prolonged exposure, leading to further deterioration of tissue integrity.

Based on these findings, both drugs exhibit toxic effects at the cellular, tissue, and systemic levels in aquatic organisms. However, Cisplatin appears to be more lethal and environmentally hazardous due to its higher toxicity, persistence, and bioaccumulation potential.

Previous studies on gastropod species have reported histopathological lesions such as increased lipid vacuolization, epithelial desquamation, glandular disruption, and inflammatory responses in mantle tissue [35]. These systematic and tissue-specific effects make Bisphenol A a suitable agent for use in positive control experiments, providing strong scientific justification for its inclusion as a control substance in this study. Bisphenol A is a widespread endocrine-disrupting compound (EDC) in aquatic environments and is commonly preferred as a positive control in laboratory-based toxicity studies. BPA is widely referenced in aquatic toxicity classification systems because it consistently induces cellular degeneration, inflammation, lipid accumulation, and reproductive impairment in various organisms [35,36,37].

The combined toxicity observed in the groups co-exposed to 5-Fluorouracil and Cisplatin suggests a potential synergistic interaction. This may result from the agents inducing DNA damage, protein synthesis inhibition, and oxidative stress through different molecular pathways. The degenerative changes observed in the mantle tissue particularly suggest that the combined exposure may exceed the physiological coping capacity of the organism.

It is well established that the histopathological responses of gastropod species to environmental toxicants enhance their potential use as bioindicators [19,31]. The sensitivity exhibited by *Melanopsis praemorsa* in this study highlights its potential as a model organism for evaluating the biological effects of pharmaceutical pollutants in aquatic environments.

5. CONCLUSION

In this study, histopathological alterations in the mantle tissue of the freshwater snail *Melanopsis praemorsa* were evaluated following chronic exposure to the antineoplastic agents 5-Fluorouracil and Cisplatin. The results indicated that the severity of tissue lesions increased in parallel with both the exposure dose and duration. Overall, the findings demonstrate that *M. praemorsa* is highly sensitive to antineoplastic compounds and, therefore, has strong potential to serve as an effective biomarker for assessing environmental toxicity in aquatic ecosystems. Furthermore, considering that the combined effects of Cisplatin and 5-Fluorouracil were more severe than their individual impacts, it is recommended that such synergistic toxicities be taken into account in environmental risk assessments.

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