Mar. Sci. Tech. Bull. (2021) 10(2): 201-206
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.887428

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Adverse effects of *Ruditapes decussatus* (Linnaeus, 1758) diet on stomach tissues in rats

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ARTICLE INFO

Article History:
Received: 26.02.2021
Received in revised form: 25.03.2021
Accepted: 30.03.2021
Available online: 06.04.2021

Keywords:
Gastritis
Heavy metal
Stomach
Cross-cut carpet shell
Ruditapes decussatus

ABSTRACT

Ruditapes decussatus (Linnaeus, 1758) belongs to the *Veneridae* family is given as nutrients to rats. *R. decussatus* was collected from three different locations from the Çanakkale Strait. Twenty-four male Wistar Albino rats (270-310 g) were used in the study. There were six rats for each experimental group. The first group fed with standard rat feed, the second group fed with 80% *R. decussatus* + 20% standard rat feed daily; the third group fed with 80% *R. decussatus* + 20% standard rat feed every other day, the fourth group fed with 80% *R. decussatus* + 80% standard rat feed every three days. After routine histopathological follow-up, gastric tissue samples of all subjects were stained and examined under the light microscope. There were no histopathological findings in the gastric tissues of rats in the control group of hematoxylin-eosin staining. In gastric tissues of rats fed with *R. decussatus*, chronic gastritis caused by mononuclear inflammation between lamina propria and occasional gastric glands was noted. It was observed that inflammation severity and distribution were high especially in tissues of rats fed with *R. decussatus* every day. It was observed that the most commonly consumed shellfish may cause pathological picture on the digestive system of rats. As a result, increasing environmental pollution threatens the life of water as well as land life, and the consumption of living organisms exposed to polluted environments continues to threaten and affect human life. It is important to pay attention to the conditions under which the consumed products are collected and how they are collected.

Please cite this paper as follows:

İrkin, L. C., Öztürk, Ş. (2021). Adverse effects of *Ruditapes decussatus* (Linnaeus, 1758) diet on stomach tissues in rats. *Marine Science and Technology Bulletin*, 10(2): 201-206.

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Introduction

Seafood is nutritionally important all over the world and there has been a significant increase in fisheries in the last two decades. Asian countries cover 90% of the aquaculture industry. In Turkey, seafood constitutes an important part of the food sector. Shellfish consumption has become widespread in Turkey coastal regions. The issue of food safety in seafood is regionally dependent on environmental conditions and production methods. The microbial quality of seafood varies greatly depending on environmental conditions, microbial quality of water, water temperature, salinity, distance from settlements, natural bacterial flora in water, food consumed by fish, fishing methods and cooling conditions (Kolsarıcı & Ertaş, 1989; Küçüköner & Küçüköner, 1990; Garret et al., 1997; Feldhusen, 2000).

Environmental pollution has been increasing day by day and threatening the lives of many living species including humans. The rapid development in the industry not only threatens the lives of land and aquatic organisms but also causes toxicity in humans who consume these organisms as foodstuffs and other aquatic organisms, which are the food source of humans (Sánchez-Marín et al., 2019). The Çanakkale Strait, an important route for trade ship crossings, has been exposed to environmental pollution for years. In fact, the Bosporus is an important region where both fish migration routes and crustaceans are cultivated. The fish and shellfish obtained here are consumed in other regions as well as the people of the region. There is not much data on the toxicity of seafood exposed to environmental pollution in other organisms. For this reason, the study was planned to determine the effects of clams which are very popular and consumed as a food source, on the stomach tissue of rats.

Ruditapes decussatus (Linnaeus, 1758) [Synonym: *Tapes decussatus* (Linnaeus 1758)] is a bivalve aquatic animal living from 1 to 12 m depth in the Çanakkale Strait (Lapseki, Çardak, Çamburnu) which belongs to *Veneridae* family. It is called Chequered carpet shell and Cross-cut carpet shell in English, Geruite tapijtschelp in Dutch, and Palourde in French. It is known that as a result of pollution in the Çanakkale Strait with environmental factors, heavy metals accumulate in its internal organs, as in other bivalves. In a study conducted on the Umurbey coast in the Çanakkale Strait about some mollusks growing in the region, zinc and manganese were found higher than the acceptable values in the clam (Gezen et al., 2011). In the sea water of the same region, the zinc level was higher than the acceptable values of heavy metals. This study aimed to reveal the histopathological effects of *R. decussatus* diet, which is delicious and has nutritious properties consumed due to human eating habits, on the rat digestive system. The aim of this experimental study is to determine the amount of heavy metals in bivalves and seawater, and compare the bioaccumulation of toxic metals in seawater and detect the relationship between heavy metal levels of bivalves and parameters of the seawater quality; to assess human health risk from heavy metal and determine the maximum amount to be consumed by humans.

Material and Methods

Chemicals and Reagents

The following chemicals were used for histopathological evaluation. 10% neutral buffered formaldehyde solution (Bio Optica, dilution: 1/7), Ethyl Alcohol Absolut (%99.8 Sigma-Aldrich, LB.SA.32221), Paraffin (MERCK, LB.M.107337.9020), xylenes (Honeywell, 16446).

Experimental Animal Design

Twenty-four male Wistar albino rats (270-300±10 g weight) were used in this study. All rats were housed in Experimental Animal (rat) shelter of Çanakkale Onsekiz Mart University, Faculty of Medicine, with an average temperature of 22±1°C, a humidity of 55±5, and a ventilation and air conditioning system for 12 hours of light and 12 hours of darkness. The rats were given as much water as they could drink. Standard rat food and *R. decussatus* were given 15% of the weight of each rat in the feeding planning.

- Group 1 (6); given standard rat feed.
- Group 2 (6); given 80% *R. decussatus* + 20% standard rat feed daily continuously.
- Group 3 (6); given 80% *R. decussatus* + 20% standard rat feed every two days; standard rat feed was given on the other days.
- Group 4 (6); given 80% *R. decussatus* + 20% standard rat feed three days apart; Standard rat feed was given for the other two days. Subjects in each group were fed for 30 days.

Histopathological Examination

Stomach tissue samples taken from all groups were detected in 10% neutral buffered formaldehyde solution (Bio Optica, dilution: 1/7) for 24 hours. The detected tissue samples were passed through graded alcohols (70%, 80%, 90%, 96%, Absolute) alcohol and their water was removed. Later, the tissues that were passed through xylene were made transparent and the tissues were removed from alcohol. Paraffin was allowed to enter into the tissue samples that were passed through xylene + paraffin and paraffin stages in a 60°C oven

(Core 500). Tissue samples extracted from paraffin were blocked using a tissue embedding device. Tissue samples taken into blocks were cut at 4-5 µm thickness in microtome for routine histopathological staining and placed in a water bath. Tissue samples opened here were taken on a normal slide and hematoxylin-eosin staining was applied.

Experiment Material Collecting and Preparing

The samples were collected from 10 to 40 m depth by scuba diving from Lapseki (40°21'6.9840" N, 26°41´31.6248" E), Çardak (40°22'45" N, 26° 42' 50" E) Çamburnu (40°10´1.1460" N, 26°22´18.0372" E). Then the samples were dried in an oven at 60 to 65 °C until a constant weight and then was ground with electric spice grinder (MRC, WSG60E) into powder. The samples were analyzed for cadmium, lead, copper and zinc by inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer Optima 8000) (Støving et al., 2013). The material used in the study was stored at -20°C. It was added to the weekly prepared feed mixture after it was dissolved.

Results and Discussion

There was no significant change in the staining of the stomach tissues of the rats in the control group (Figure 1) with hematoxylin-eosin staining. Tunica mucosa, submucosa, lamina propria and muscularis mucosa layers were observed in the normal histological structure. There were signs of active chronic gastritis in the gastric mucosa of rats in the second group (Figure 2). It was observed that the inflammation was distributed between the lamina propria and gastric glands. Among the groups, the most severe inflammation table was seen in this group. While chronic gastritis was observed in the third group (Figure 3), inflammation was observed to be less severe, and mononuclear inflammatory cells were observed rarely in the fourth group (Figure 4). In the semi-quantitative scoring made in terms of inflammation, it was seen that the meaning of gastritis increased in the second group compared to the other groups.

Heavy Metal Analysis

According to the results, considering the Cadmium (Cd), Copper (Cu), Lead (Pb), and Zinc (Zn) level in *R. decussatus* the location where the heavy metal concentration is high are Lapseki and Çardak (Table 1). As heavy metal analysis results of the seawater from which the clam samples were taken, the values of the Lapseki and Çardak region were found to be in parallel with the heavy metal values in the muscle tissue of the clam samples obtained from these regions. Heavy metal accumulation in clam tissue suggests that it may trigger toxicity in tissues with the consumption of marine based food frequently (Table 1).

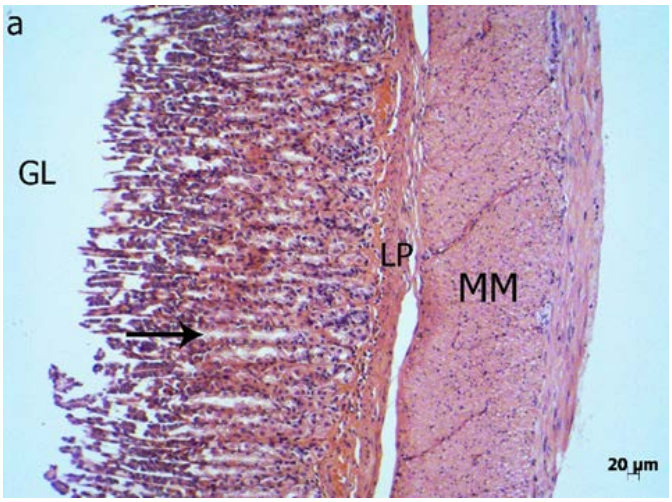

Figure 1. Group 1 Hematoxylin-Eosin staining of the gastric mucosa (X10) (GL: Gastric lumen, LP: Lamina propria, MM, Muscularis mucosa, arrow: Gastric pit)

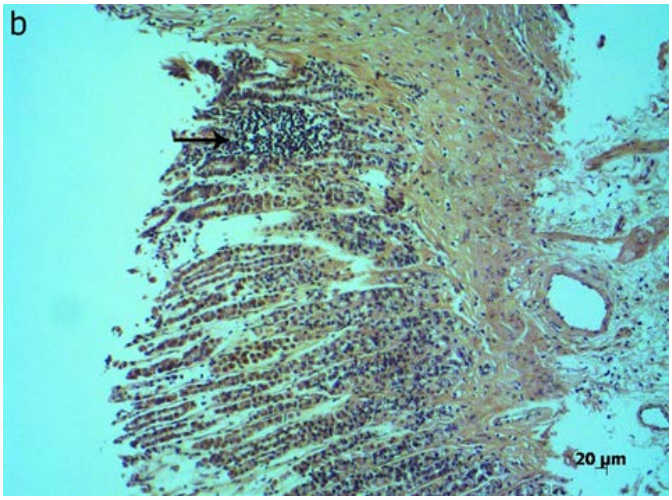

Figure 2. Group 2 Hematoxylin-Eosin (X10) (Arrow: inflammation in the lamina propria)

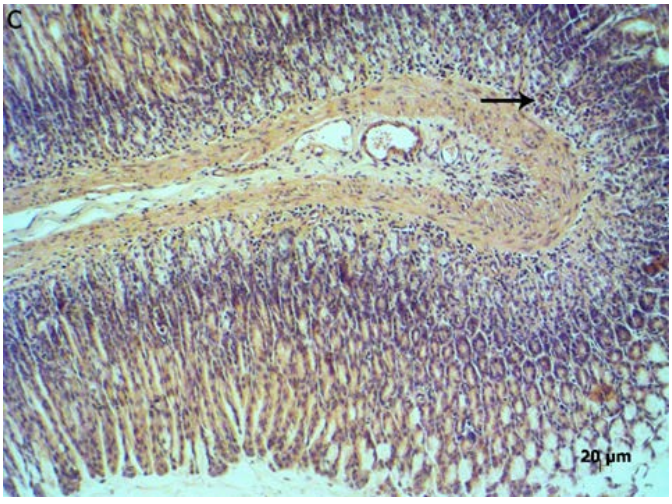

Figure 3. Group 3 Hematoxylin-Eosin staining (X10) (Arrow: inflammation)

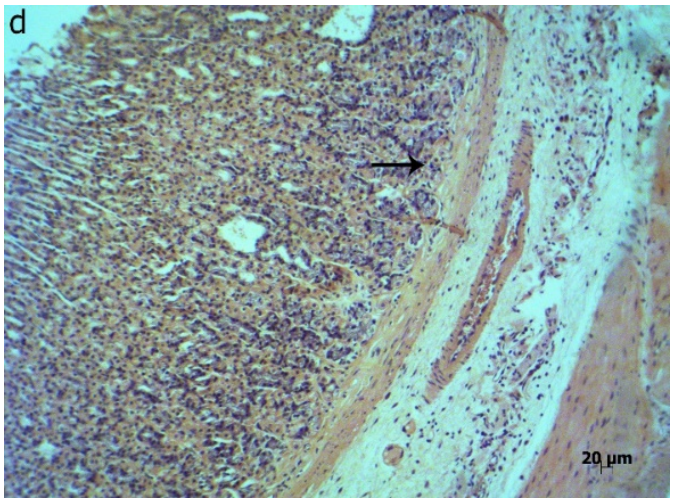

Figure 4. Group 4 Hematoxylin-Eosin staining (X10) (Arrow: inflammation)

As a result of the analyzes, it was determined that Cd, Cu, Pb, and Zn values in sea water were higher than the upper limit values determined by the Turkish Standards Institution (TSI). Cd, Cu, Pb, and Zn levels in sea water samples were found to be higher in the Çardak region and Zn in the Çamburnu region. The upper limit values determined by the Turkish Standards Institution and the World Health Organization are shown in Table 2.

Currently, there are no adequate medical epidemiological studies on the negative effects of marine pollution on human health (Allen, 2011). Various studies have shown that there is an accumulation of elements such as copper, nickel, zinc and lead in cancerous tissue samples (Yaman et al., 2007). Free radicals are electron acceptor molecules of the biological system. Ustunada et al. (2011) determined the values of the metals such as Cu, Zn, Pb and Cd in *C. fragile* at the highest level, respectively. Algae are among the foods consumed in many parts of the world, especially as a salad. They can produce different effects in organisms that consume depending on the pollution of their environment such as sand mussels and other sea creatures. Especially heavy metal accumulation is an inevitable fact in aquatic organisms. Crustaceans have a good filter system and feed by a filtering system. It has a high potential for heavy metal deposition. In the present study, it was found that the amount of lead accumulated in bivalves *in vivo* is quite high (Sánchez-Marín et al., 2019). *In vivo* experimental study shows that cadmium poisoning causes a serious degeneration in kidney epithelial cells in addition to the expansion and adhesion of secondary lamellae in the gills, as well as in fish fed with cadmium-contaminated fish food (Beširović et al., 2011). These studies show crustaceans that store toxic products increase oxidative stress parameters and trigger apoptosis, such as bacteria, infectious agents, and heavy metal, and can pass to other organisms after consumption. In this study, it was determined that the crustacean consumption of the rats was two weeks and 20%, therefore fewer toxic values were found than our findings. However, toxic effects were found to be considerably high in the stomach tissue of rats that we fed with long-term and shelled feed.

Table 1. Heavy metal concentrations of *R. decussatus* muscle tissue (µg/g dry weight, *Turkish Food Codex, **World Health Organization)

Heavy metals and Region	Cd	Pb	Cu	Zn
Camburnu	1.32±0.23	0.68±0.45	1.47±0.28	20.74±2.6
Lapseki	1.54±0.5	0.81±0.47	1.53±0.3	23.74±3.87
Cardak	0.94±0.24	0.45±0.38	0.82±0.33	18.24±4.22
Average value	1.26±0.32	0.64±0.43	1.27±0.3	20.90±3.56
Limit value (µg/g) **WHO	1	1	5	20
Limit values (mg/L) *TFC	0.5	0.5	3	10

Table 2. Heavy metal analysis of seawater for different region in Çanakkale Strait (*Turkish Standards Institution, **World Health Organization, United States Environmental Protection Agency)

Heavy metals (µg/L)	Region			Average value	Limit values (mg/L) *TSI	Limit values (mg/L) **WHO and USEPA
	Çamburnu	Çardak	Lapseki			
Cu	1.94±0.28	2.24±0.65	1.88±0.54	2.02±0.49	0.01	0.01
Cd	1.75±0.28	1.72±0.45	1.74±0.66	1.73±0.46	0.01	0.01
Pb	0.85±0.22	0.86±0.35	0.94±0.44	0.88±0.36	0.1	0.10
Zn	40.14±3.84	42.62±4.02	42.35±5.20	41.70±4.35	0.1	0.10

Active oxygen derivatives are called oxidants (Erken, 2012). They cause cell death by causing protein modifications, lipid peroxidation and DNA fragmentation (Bakonyi and Radak, 2004). It is known that there are different defense mechanisms against free radicals such as repair, physical defense, and antioxidant defense. A small part of the reactive oxygen varieties produced in the body escape from the antioxidant defense system and cause some systemic diseases and aging (Berger, 2005). Heavy metals and infectious agents that pass into tissues with foods disrupt the oxidant/antioxidant balance and cause cell death along with oxidative stress. In this study, it has been shown that excessive consumption of *R. decussatus* can cause serious damage to the rat digestive system, especially these creatures that are not supplied from a clean environment.

The data obtained in this study revealed that *R. decussatus* grown in Lapseki-Çardak region of the Çanakkale Strait may cause histopathological changes in digestive system of organisms fed with these animals frequently. Because of it should be ensured that water resources are kept clean. The number of researches on aquatic organism in polluted waters should be increased and these foods obtained from areas considered to be contaminated should be consumed after a strict control.

Conclusion

It is possible that crustaceans can transmit harmful factors such as heavy metals, pesticides, viral and bacterial organisms collected by filtering from their diet to the mammals consuming these animals. However, in vivo studies are scarce in the literature. Therefore, the findings obtained from the present study may shed light on experimental diet studies. It is required to make a more comprehensive study, to develop concurrent nutritional models and to obtain clinical results. An experimental feeding model will provide an idea about whether the histopathological findings that we think may be acute will cause chronic disease. The scarce of the available reports caused limitations in our interpretation of the clinical picture to compare the findings. Food safety has a great importance for health. Habitat conditions in which aquatic organsims consumed as food sources obtained are important. For this reason, importance should be attached to the studies determining environmental pollution and international measures should be increased on what can be done to eliminate the negative effects caused by this situation.

Compliance with Ethical Standards

Authors' Contributions

Author LCİ designed the study. ŞÖ and LCİ wrote the first draft of the manuscript, LCİ and ŞÖ performed and managed statistical analyses. Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

A total of 24 male Wistar albino rats were used in the study. The study protocol was approved by the Çanakkale Onsekiz Mart University Ethics Committee for Animal Research (Protocol number: 2021/02-01).

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