

HISTOPATHOLOGICAL CHANGES IN THE STOMACH MUCOSA OF RATS FED WITH GREAT SCALLOP (*Pecten maximus*)

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ARTICLE INFO	ABSTRACT
<p>Article History: Received: 05 June 2018 Accepted: 11 July 2018</p>	<p><i>Heavy metals, industrial and household wastes and pesticides are threats for the aquatic ecosystem. Polluted water sources are streaming into the seas and cause pollution in these systems. Dardanelles is exposed to pollution from the Marmara and Black Sea. Our previous studies demonstrated that the water and mollusc from certain regions of the Dardanelles contained heavy metal salts. The purpose of the study is to demonstrate the histopathologic changes in the gastric tissues of rats which are fed with great scallop (<i>Pecten maximus</i>) that are collected from the Çardak region of the Dardanelles. Four groups of rats are included in the study, group 1 (n=6), control group fed with standard rat food, group 2 (n=6), 75% great scallop and 25% standard rat food daily, group 3 (n=6), 75% great scallop and 25% standard rat food every two days, group 4 (n=6), 75% great scallop and 25% standard rat food every three days. After the routine histopathologic processing all gastric tissue samples are evaluated in terms of 8-hydroxy-2'-deoxyguanosine (8-OHdG) immunoreactivity with light microscopy and image analysis software. No histopathologic differences found in standard hematoxylin-eosin (H.E.) stained gastric tissue samples of the control group. Second group showed active chronic gastritis, third group showed less inflammation and chronic gastritis compared with the second group and fourth group showed less mononuclear inflammation compared to the second and third groups. In immunohistochemical staining, 8-OHdG immunoreactivity in gastric epithelial cells. 8-OHdG immunoreactivity was negative in stomach tissues in all groups. There was no statistically significant difference between the groups that were fed every day, every other day and every three days with great scallop ($p>0.05$). The results of our studies showed that rats fed more with great scallops could produce gastritis in the stomach.</i></p>
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1. INTRODUCTION

Sea pollution is an indispensable part of environmental pollution. Domestic and industrial wastes, nuclear power stations built for electric generation, erosions, improper coastal fill areas, oil pollution, and marine accidents are significant factors causing sea pollution (Topcuoğlu et al., 2003; Tüzen 2003).

Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration and substrate oxidation.

Small amounts of ROS, including hydroxyl radicals (OH), superoxide anions (O₂) and hydrogen peroxide (H₂O₂), are constantly generated in aerobic organisms in response to both external and internal stimuli (Hurst et al 1997; Jornot et al., 1998). Low levels of ROS are indispensable in many biochemical processes, including intracellular messaging in the cell differentiation and cell progression or the arrest of growth, apoptosis (Ghosh et al. 1998), immunity (Yin et al. 1995) and defense against micro-organisms (Bae et al. 1997; Lee et al. 1998). In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological macromolecules (Chopra et al., 1998; Czene et al., 1997; Wojtaszek 1997).

Under normal conditions, the proportion of heavy metals in the environment is low. When the concentration ratio in the natural environment increases, heavy metals such as silver, mercury copper, cadmium and lead are toxic on organisms and inhibit enzymes. For some enzymatic activities in the living world, certain metals are necessary, provided they are in certain concentrations. The metals bound to the organic material can be used during biological activities and dissolve again by dissociation of the organic materials (Balkıs et al., 2005). Estimates of intake of these elements were made through seafood consumption by the general population Turkish legal standards, are 1.0 ppm for Cd and 2.0 ppm for Pb in bivalve molluscs (Anonymous 2000).

Heavy metals were detected in seawater and many molluscan species that growing in the Dardanelles (Gezen et al., 2011; Demir et al., 2011; Gezen et al., 2011; Özkurnaz et al., 2012). The accumulation of heavy metals have investigated in the carpet shell clams, great scallops, sea snails and oysters from the Dardanelles Umurbey region. In this research, Zn in carpet shell clams, Zn and Mn in great scallops, Zn in oysters, Al, Zn, Fe, Cu and Mn in sea snails found the metals as high. If the same zone is in seawater, the Zn level is high (Gezen et al., 2011). In sea chestnuts growing in Dardanelles, the values of Al, Zn, and Fe in samples taken from Gelibolu Hamzakoy station are high. Al and Fe values were higher in samples taken from Çardak region. Al, Fe and Zn values were higher in samples taken from Umurbey region. Al, Fe and Zn values were higher in samples taken from Çamburnu region (Gezen et al., 2011). The accumulation of heavy metals have investigated in the carpet shell clams, great scallops, sea snails and oysters from the Dardanelles Karacaören region. In this research, Al, Zn and Fe in carpet shell clams, Zn and Mn in great scallops, Zn in oysters, Al, Zn, Fe, Cu and Mn in sea snails found the metals as high (Demir et al., 2011).

There is no research revealing histopathologic changes in the stomach tissues of living beings fed with great scallops collected from the Çardak region (Çanakkale, Turkey). The purpose of the study is to demonstrate DNA damage and the histopathologic changes in the gastric tissues of rats which are fed with great scallops that are collected from the Çardak region (Çanakkale, Turkey).

2. MATERIAL AND METHODS

2.1. Ethics Statement

The study protocol was approved by the Çanakkale Onsekiz Mart University Ethics Committee for Animal Research (Protocol number: 2010/09-03).

2.2. Animal Model

A total of 24 male Wistar albino rats, weighing 290–310 g, were used in the study. The rats were kept for 30 days under appropriate conditions of temperature/humidity and a 12-h light cycle while being provided sufficient water and feed. The rats were randomly selected and divided into 4 groups. For the first study group (n: 6), was the control group; standard rat diet was given every days. For the second study group (n: 6), 75% great scallop +

25% standard rat diet standard rat feeds were given daily. For the third study group (n: 6), 75% great scallop + 25% standard rat diet was given every two days. Standard rat diet was given the other day. For the fourth group (n: 6), 75% great scallop + 25% standard rat diet was given every three days. Standard rat diet was given the other two day.

Rats were fed twice daily for 30 days at 15% of their weight every morning and evening at the same time. The great scallop given as food to the rats were removed from the Dardanelles Çardak region (Figure 1). Average 40-60 g weight were selected. After the beaks were overcooked, the meat broke off and the meat at 100 degrees was dried.

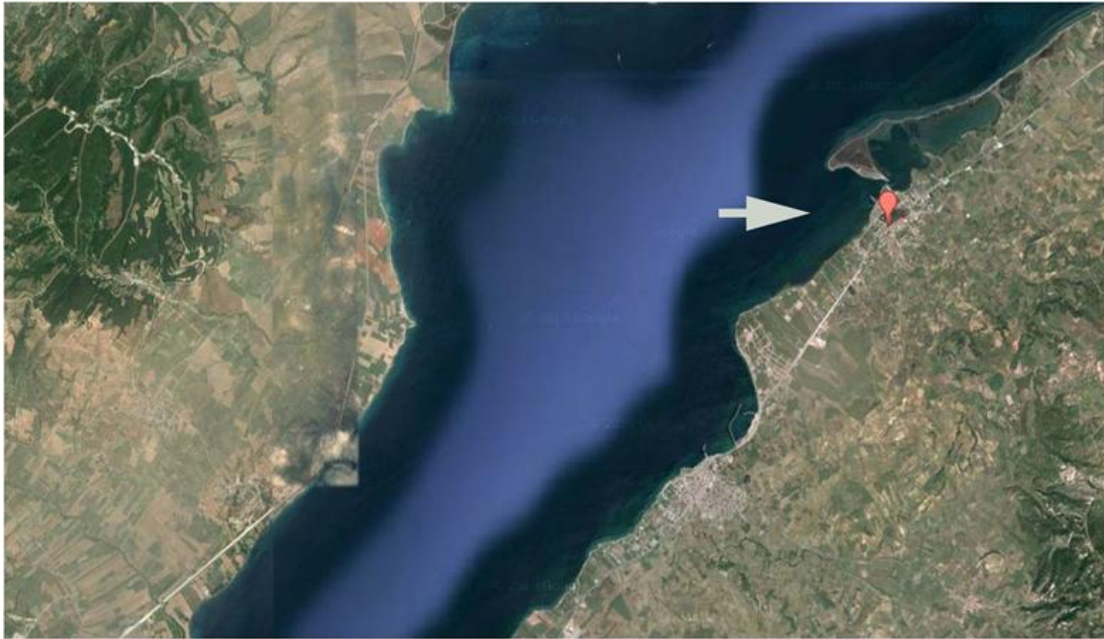


Figure 1: Dardanelles Çardak region (Çanakkale, Turkey). **Arrow:** The area where the great scallops are collected.

It was weighed into each rat's weight and 10 mg/kg intraperitoneal ketamine hydrochloride (Ketalar, Eczacıbasi, Istanbul, Turkey), and 20 mg/kg of xylazine 2% (Rompun, Bayer Turkey Pharmaceutical Ltd., Istanbul, Turkey) were anesthetized. The rats were anesthetized and then sacrificed.

2.3. Histological evaluation

The stomach tissues were maintained in immunofix (Leica) for 24 hours for histopathological examination. The paraffin embedded stomach tissues were stained with hematoxylin and eosin (H & E) at a thickness of 5 microns. Immunohistochemical staining method was applied by cutting the paraffin embedded stomach tissues 3 microns in thickness.

The LAB-SA Detection System, (Histostain-Plus Bulk Kit, Invitrogen) was applied to determine immunohistochemical localization of 8-hydroxydeoxyguanosine (8-OHdG) in tissues. Slides were incubated with polyclonal goat anti-8-Hydroxydeoxyguanosine (8-OHdG, Millipore Corporation) antibody, which was diluted 1:200 for the stomach tissue, for 30 minute at room temperature. Diaminobenzidine-tetra hydrochloride (DAB, Invitrogen Corporation) was used as the colorant. Also Mayer's hematoxylin stain was used as a nuclear counterstain.

Dye samples were evaluated on the Zeiss AXIO Scope 1 brand research microscope. Analysis of 8-OHdG immunoreactive cells in the stomach tissue was performed using the Leica LAS V3.8 image analysis system. Five of the sections from the blocks containing the stomach tissues of all the rats in all groups were stained. From the stained sections, 1000 cells were counted and immunoreactive cells were identified among these cells. Tosun et al., (2006); Bakır et al., (1996); Avunduk et al., (2000) for this purpose;

Immunopositive cells / Total cell count (1000) ----- x 100 % = % formula were used .

2.4. Statistical analysis

SPSS 15 version was applied for the statistical evaluation of the results obtained with the applied formula. Kruskal-Wallis Test was used for nonparametric tests to determine the differences between survivin immunoreactivity groups. The difference between the groups was considered significant in the results of $p < 0.05$.

3. RESULTS

There was no significant change in the staining of the stomach of the rats in the first group with Hematoxylin Eosin (Figure 2).

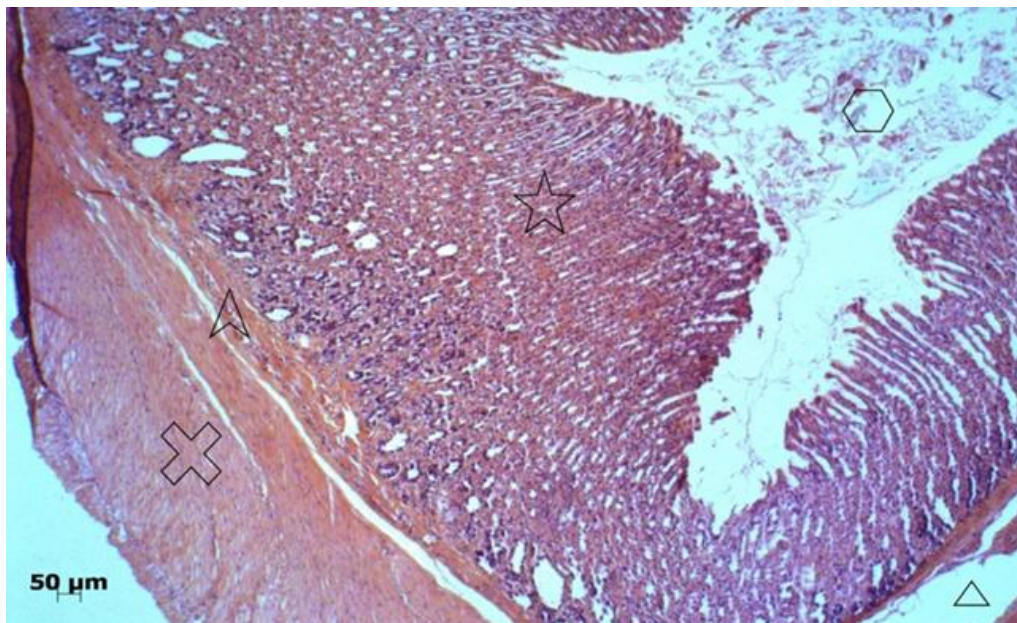


Figure 2. For the first study group was the control group; standard rat diet was given every days. Rat stomach, (H.E.x5). **Star:** Lamina propria mucosa; **Pointed arrow:** Lamina muscularis mucosa; **Arrow head:** Lamina submucosa; **Crossed:** Tunica muscularis; **Hexagon:** Gastric lumen.

In the second group, there were active chronic gastritis findings in the rat gastric mucosa (Figure 3). Mononuclear inflammatory cells were observed as foci between the lamina propria and the border of the lamina muscularis. Besides, common mononuclear cells were detected in lamina propria mucosa, lamina muscularis mucosa and lamina submucosa.

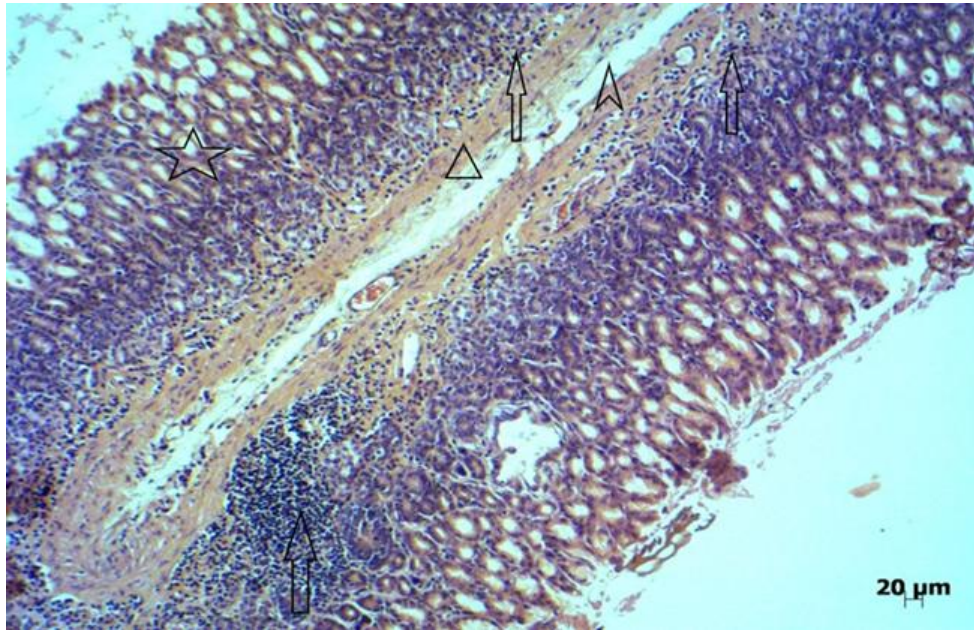


Figure 3. For the second study group; 75% great scallops + 25% standard rat diet standard rat feeds were given daily. Rat stomach, (H.E.x10). **Star:** Lamina propria mucosa; **Arrow head:** Lamina muscularis mucosa; **Pointed arrow:** Lamina submucosa; **Arrows:** Mononuclear inflammatory cells

In the third group, mononuclear inflammatory cells were observed in some areas of the lamina muscularis mucosa, more in the lamina propria mucosa (Figure 4).

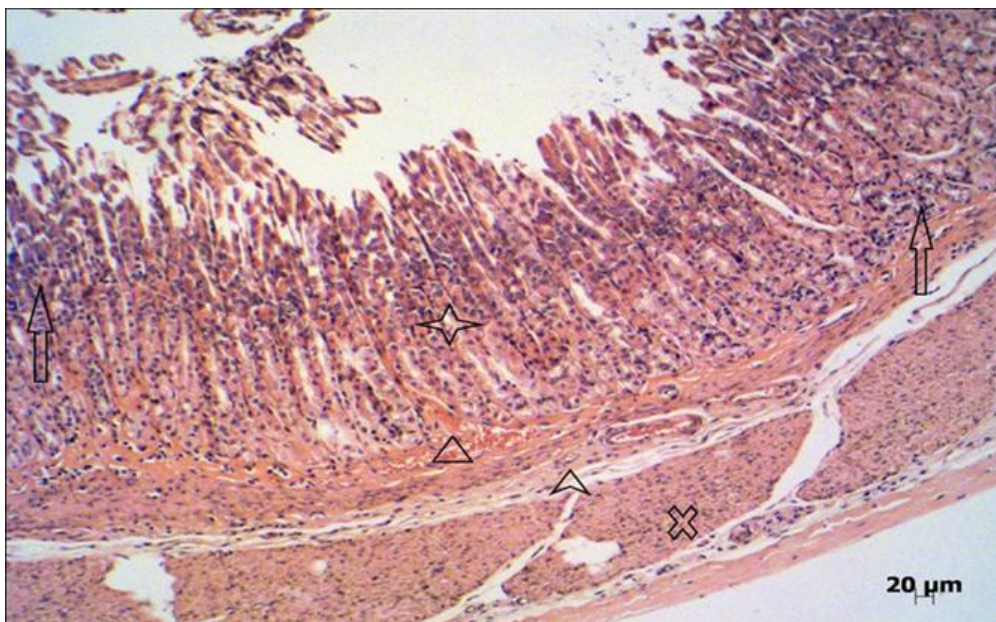


Figure 4. For the third study group; 75% great scallops + 25% standard rat diet was given every two days. Standard rat diet was given the other day. Rat stomach, (H.E.x10). **Star:** Lamina propria mucosa; **Arrow head:** Lamina muscularis mucosa; **Pointed arrow:** Lamina submucosa; **Crossed:** Tunica mucosa; **Arrow:** Mononuclear inflammatory cells.

In the fourth group, mononuclear inflammatory cells were rarely seen in some areas of the lamina muscularis mucosa, more in the lamina propria mucosa (Figure 5).

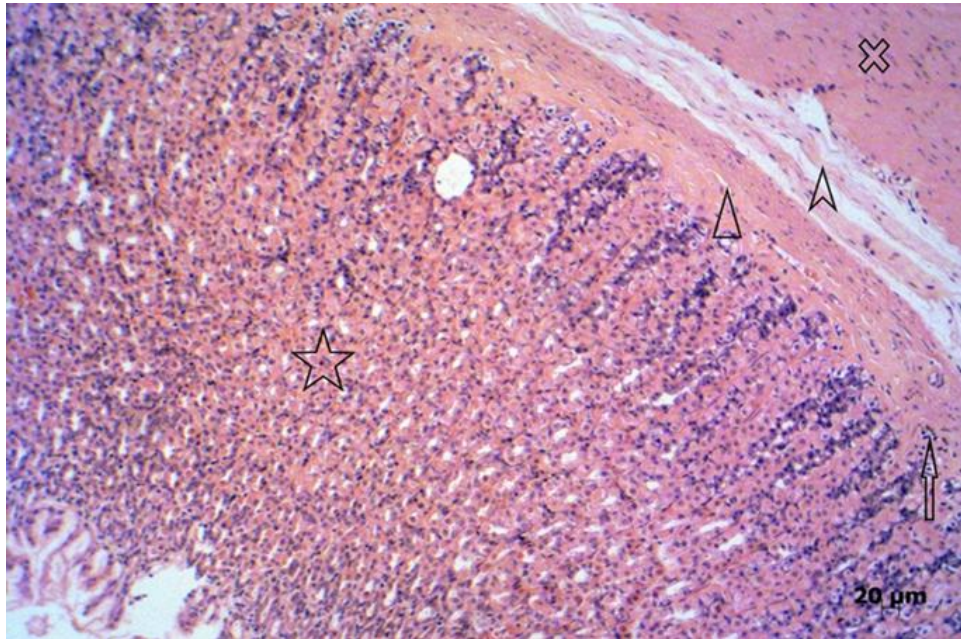


Figure 5. For the fourth group; 75% great scallops + 25% standard rat diet was given every three days. Standard rat diet was given the other two day. Rat stomach, (H.E.x10). **Star:** Lamina propria mucosa; **Arrow head:** Lamina muscularis mucosa; **Pointed arrow:** Lamina submucosa; **Crossed:** Tunica muscularis; **Arrows:** Mononuclear inflammatory cells

8-OHdG immunoreactivity was negative in stomach tissues in all groups. No significant differences were detected between groups in immunohistochemical staining with 8-OHdG (Figure 6).

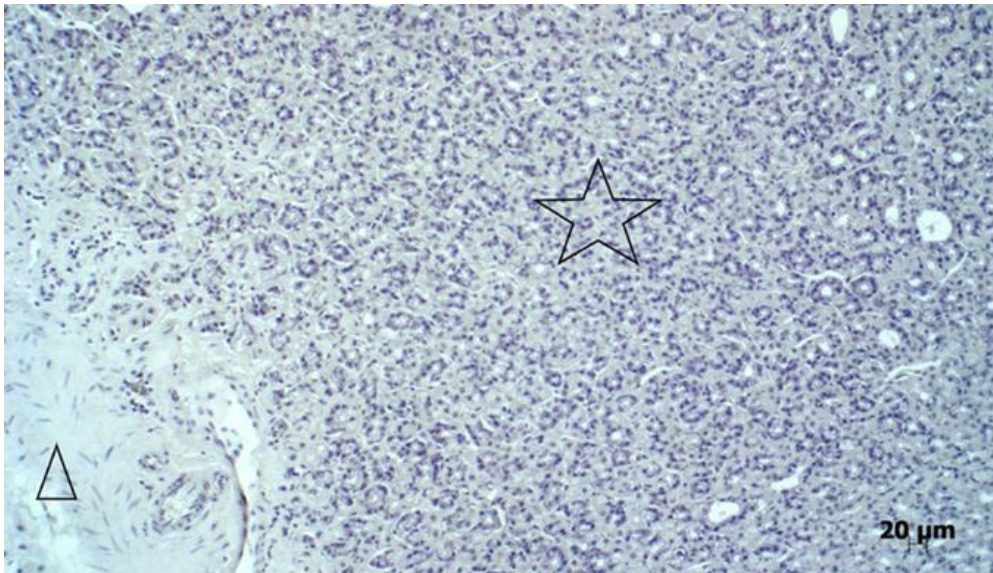


Figure 6. For the second study group; 75% great scallops + 25% standard rat diet standard rat feeds were given daily. Rat stomach. (8-OHdGx10). **Star:** Lamina propria; **Arrow head:** Lamina muscularis.

4. DISCUSSION

Extensive mononuclear cell infiltration was detected in the stomach of all rats consuming great scallops every day for 30 days, especially in the lamina propria mucosa, with foci of mononuclear inflammation and in all mucosa layers. Extensive mononuclear cell infiltration was detected in tunica mucosa layers of gastric tissue of rats consuming great scallops every other day for 30 days. Mononuclear cell infiltration was detected in the tunica mucosa layers of the stomach of the rats consuming mussels every three days for 30 days.

Cadmium, which is a highly toxic metal, causes necrosis by accumulating especially in liver and kidney (Kara et al., 2004). It has been revealed that heavy metals can cause chronic degenerative changes and, in some cases, can cause teratogenic and carcinogenic effects, especially by affecting the nervous system, liver and kidneys (IARC 1987). In addition to the findings of other researchers, we have also found that heavy metal salts cause histopathological changes in the stomach tissue.

Long-term exposure to heavy metals such as mercury (Hg), lead (Pb), chromium (Cr), cadmium (Cd), arsenic (As), copper (Cu), vanadium (V), nickel (Ni) chronic inflammation, cardiac, pulmonary and neurological effects and some cancers (Nieboer et al., 2013; Mantovani et al., 2008; Clarkson, 2002). In this study, we found mononuclear inflammatory cell growth and chronic gastritis in gastric mucosa of rats fed with great scallops. Findings by some investigators that heavy metals may cause inflammation support our findings.

Some researchers have found that heavy metal salts can cause DNA damage (Fraga et al., 1990; Halliwell et al., 2000; William et al., 2000; Ateş et al., 2004; Burçak et al., 2004; Siomek et al., 2006). Immunohistochemical staining methods are used to detect damage to cells and tissues (Gezen 2017). 8-OHdG was found to be negative in the gastric mucosa of all groups in our study. We could not detect DNA damage in the stomach tissue of rats fed with great scallops. It is thought that heavy metals in low levels (NOEL) in the grains do not cause DNA damage.

However, increased inflammation in the stomach mucosa suggests that digestive system diseases have arisen. The authors think that care must be taken while consuming this kind of great scallops.

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Footness

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