iNOS Immunoreactivity in the Gastric Mucosa of Rats Feedind with Mussels (*Mytilus galloprovincialis*)

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Abstract

The mussels are living things that collect the pollutants in sea water and filter the sea water. Although there are few data about the toxicity of seafood that is exposed to environmental pollution, there are no animal studies about the gastric toxicity of mussels grown in the Dardanelles. The purpose of the study is to demonstrate the inducible nitric oxide synthase (iNOS) immunoreactivity in the gastric tissues of rats which are fed with mussels that are collected from the Çamburnu region of the Dardanelles. For this purpose, four groups (n = 6) of rats were included in the study. The control group received standard rat feed every day. The second group was given 75% mussels every day and 25% standard rat diet. The third group received a 75% mussel and 25% standard rat diet every two days. The fourth group was given 75% mussels and 25% standard rat diet every three days. iNOS immunoreactivity was detected 0% in control group, 29.17% in the second group, 28.83% in the third group and 13.33% in the fourth group. There was statistically significant difference between the iNOS immunoreactivity of epithelial cells in the gastric mucosa of the rats in the experimental and control groups ($p > 0.05$). Detection of iNOS in the gastric mucosa of rats fed with mussel that we use in our work suggests that it may promote digestive system diseases.

Keywords: Mussels, Dardanelles, iNOS, Immunohistochemistry, Stomach.

1. Introduction

Heavy metals are natural components of the earth's crust and as such are the oldest toxins known to humans, having been used for thousands of years. Potential exposures to heavy metals include natural sources (eg, groundwater, metal ores), industrial processes, commercial products, folk remedies, and contaminated food and herbal products. Virtually all heavy metals are toxic in sufficient quantities. Several, however, are of particular interest because of their concentrations in the environment (lead, mercury, and arsenic) or their use in criminal poisonings (arsenic and thallium). Entering our bodies by way of food, drinking water, and air, metals producetoxicity by forming complexes with cellular compounds containing sulfur, oxygen, or nitrogen. The complexes inactivate enzyme systems or modify critical protein structures leading to cellular dysfunction and death. The most commonly involved organ systems include central nervous, gastrointestinal (GI), cardiovascular, hematopoietic, renal, and peripheral nervous systems. The nature and severity of toxicity vary with the heavy metal involved, its exposure level, chemical and valance states (inorganic versus organic), mode of exposure

(acute versus chronic), and the age of the individual (Ibrahim et. al., 2000). IARC, (1987) has explained that heavy metals may affect and cause chronic degenerative changes and, in some cases, teratogenic and carcinogenic effects, especially by affecting the nervous system, liver and kidneys.
In our previous research have investigated the accumulation of heavy metals in the carpet shell clam, clam, sea snails and oysters from the Dardanelles Umurbey region. In this research, Zn in carpet shell clams, Zn and Mn in clams, 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 Dardanells, 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). In our previous research have investigated the accumulation of heavy metals in the carpet shell clam, clam, sea snails and oysters from the Dardanelles Karacaören region. In this research, Al, Zn and Fe in carpet shell clams, Zn and Mn in clams, Zn in oysters, Al, Zn, Fe, Cu and Mn in sea snails found the metals as high (Demir et. al., 2011).
The mechanism of toxicity of some heavy metals still remains unknown, although enzymatic inhibition, impaired antioxidants metabolism, and oxidative stress may play a role. Heavy metals generate many of their adverse health effects through the formation of free radicals, resulting in DNA damage, lipid peroxidation, and depletion of protein sulfhydryls (e.g., glutathione) (Valko et. al., 2005). Although not fully proven, Al accumulation in the brain is proposed to be associated with neurodegenerative diseases, including Alzheimer's dementia, Parkinson's disease, amyotrophic lateral sclerosis, and dialysis encephalopathy (Gonçalves and Silva, 2007). Nutritional status is another significant risk factor for Pb intoxication and its effects. Iron, zinc and calcium deficiencies increase the retention of ingested Pb, which can also increase Pb gastrointestinal absorption (Goyer, 1996; Ruff et. al., 1996), and affect the susceptibility to Pb neurotoxicity (Aimo and Oteiza, 2006). Human exposure to Al is mainly caused by environmental factors, such as soil contamination (Yokel et. al., 2008). Al absorption are the gastrointestinal tract (Ittel, 1993).
Minerals play a critical role in İNOS expression. High levels of copper increase iNOS expression in lung, liver, and aorta (Cuzzocrea et. al.,2003), thus demonstrating that exessive copper can have detrimental effects on both constitutive and inducible NO synthesis. The combined effects of increased iNOS expression and decreased eNOS activity in the same anatomical location could have profound consequences on inflammatory processes of cells within the cardiovascular bed. Iron either increases iNOS expression in macrophages and proximal tubules (Chen et. al., 2001) or supresses elevated iNOS protein levels in the hearth and kidney (Ni et.al., 1997). Thus, iron excess or deficiency can impair immunlogical, cardiovascular, and renal function. In addition, zinc modulates iNOS expression in the small intestine, thereby preventing cytokine-induced diarrhea (Cui et. al., 1997). In addition, chromium and lead inhibit, but nickel and cobalt incresae, iNOS expression in activated macrophages (Tian and Lawrence 1997), suggesting an important role for NO in mediating the cytotoxic effects of environmental contamination by metals.
We could not find any research about the changes that the living creatures that consumed the mussel growing in the contaminated water could bring about in the digestive system. Mussels are a seafood that is consumed frequently by our people. A large part of the mine consumed in our country is collected from Dardanelles. The purpose of the study is to demonstrate iNOS immunoreactivity in the gastric tissues of rats which are fed with mussels that are collected from the Çamburnu region (Çanakkale, Turkey).

2. Material And Method

2.1. Ethics Statement

A total of 24 male Wistar albino rats, weighing 290-310 g, were used in the study. The study protocol was approved by the Çanakkale Onsekiz Mart University Ethical Counsil of Animal Research (Protocol number- 2010/09-03).

2.2. Animal Model

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% mussels + 25% standard rat diet standard rat feeds were given daily. For the third study group (n: 6), 75% mussels + 25% standard rat diet was given every two days. Standard rat diet was given the other day. For the fourth group (n: 6), 75% mussels + 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 mussels given as food to the rats were removed from the Dardanelles Çamburnu region (Figure 1). Average 100±10 g weight were selected. After the beaks were overcooked, the meat broke off and the meat at 100 degrees was dried.

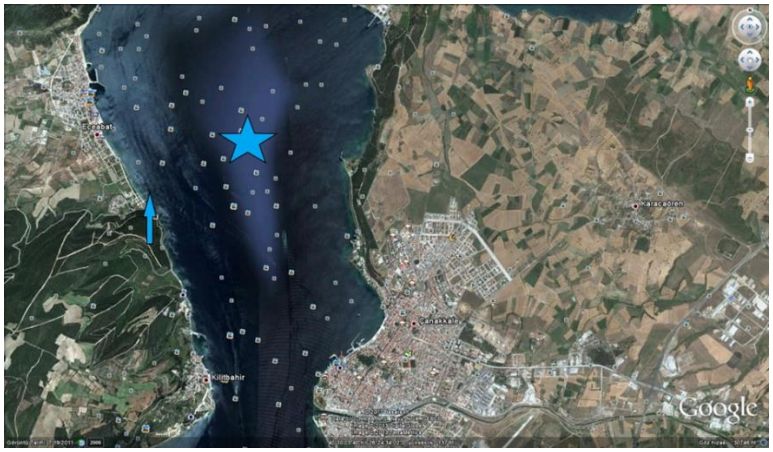

Figure 1. *The area where the mussels are collected.* ***Arrow:*** *Çamburnu region (Çanakkale, Turkey),* ***Star:*** *Dardanelles*

It was weighed into each rat's weight and 10 mg/kg intraperitoenal ketamine hydrochloride (Ketalar, Eczacibasi, 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. After the rats have received the stomachs other organs were also taken for further research.

2.3. Histological evaluation

The stomach tissues were maintained in immunofix (Leica) for 24 hours for histopathological examination. The tissue was routinely subjected to histopathological procedures and blocked. 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 iNOS enzyme in tissues. Sections taken from paraffin blocks were deparaffinized and rehydrated. Subsequently, tissue samples were resuspended in 0. 2% Triton X 100 (Santa Cruz Biotechnology) solution prepared with Phosphate Buffer Saline (PBS, Invitrogen) for 5 min. were kept. This allowed better passage of solutions from the pores in the cell and nucleus membranes. The tissue samples confined to the Pap pen were washed three times with PBS for 3 min. Subsequently, 3% H_2O_2 was applied to the sections to block endogenous peroxidase activity. The sections were incubated in citrate buffer (0. 1 M, pH: 6. 0) in the microwave (800 watt, 10 min) for antigen retrieval, and the samples were washed with phosphate buffer solution (PBS, 0. 1 M, pH 7. 2). After the samples had been incubated in the blocking buffer for 10 min, they were washed with PBS. Next, slides were incubated with anti-iNOS (inducible nitric oxide synthase) antibody (anti-NOS2, Santa Cruz Biotechnology), which was diluted at 1: 400 for the stomach tissue, for an hour at room temperature, and they were then washed with PBS. Afterwards, biotinylated secondary antibody was applied to the samples for 30 min (Ultravision Detection System, Thermo Scientific, Fremont, USA). Then the samples were washed with PBS again and incubated with Broad Spectrum Antibody (Invitrogen, USA) for 30 min. After washing the samples, diaminobenzadine-tetrahydrochlorid (DAB, Invitrogen Corporation) was applied to them. Negative control was used to determine specific iNOS immunoreactivity, and hematoxylin stain was used as a nuclear counter stain.
Dye samples were evaluated on the Zeiss AXIO Scope 1 brand research microscope. Analysis of iNOS 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. For this purpose;
Immunopositive cells / Total cell count (1000) X 100 % = ……..….. % formula were used (Bakır et. al., 1996; Avunduk et. al., 2000).

2.4. Statistical analysis

Data was analyzed using SPSS program, version 19.0 One-way analysis of variance (ANOVA), Tukey's test was used to analyze the data. The difference between the groups was considered significant in the results of $p < 0.05$.

3. Results

In immunohistochemical staining with iNOS, a significant difference was observed in the gastric epithelial cells of the rats given mussels per day, every other day and every three days compared to rats fed with normal feed ($p<0.05$).
Dark brown staining in the cytoplasm of the cells was considered positive. iNOS immunoreactivity could not be detected in gastric mucosa cells of rats fed standard rat diet (Figure 2).

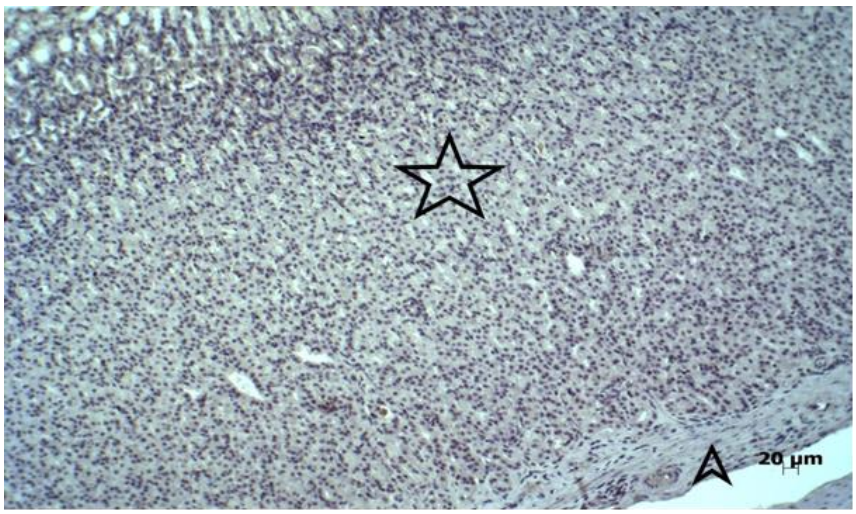

Figure 2. *For the first study group was the control group; standard rat diet was given every days. Rat stomach, (iNOS x 10).* ***Star:*** *Lamina propria mucosa,* ***Pointed arrow:*** *Lamina muscularis mucosa*

In the second group, in the surface epithelial cell cytoplasm and stomach gland cells cytoplasm showed iNOS immunoreactivity. In the superficial mucous cell cytoplasm, isthmus and neck mucous cells, the iNOS immunoreactivity was mild, whereas the cytoplasm of the cells in the basal region was strongly stained (Figure 3).

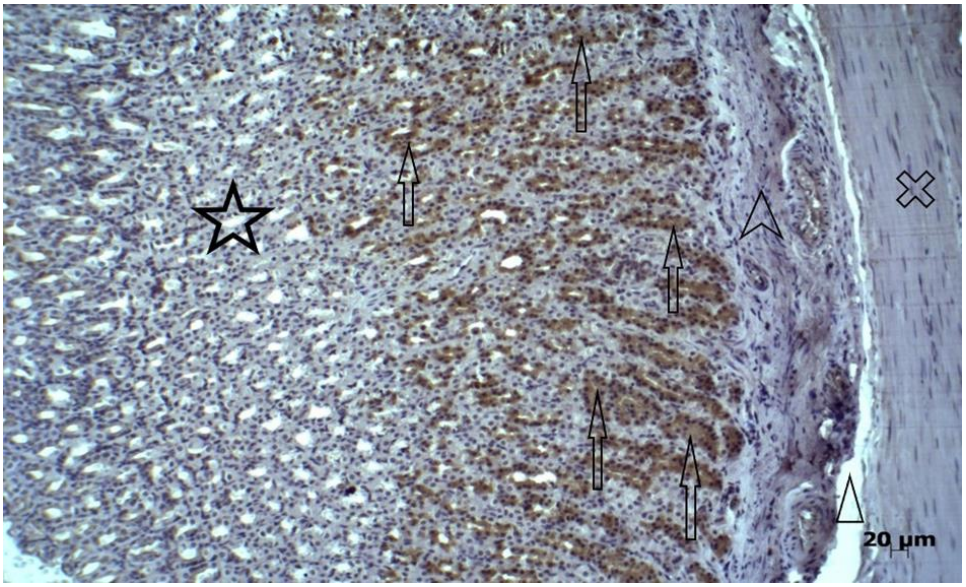

Figure 3. *For the second study group; 75 mussels + 25% standard rat diet standard rat feeds were given daily. Rat stomach, (iNOSx10).* ***Star:*** *Lamina propria mucosa,* ***Pointed arrow:*** *Lamina muscularis mucosa,* ***Arrow head:*** *Lamina submucosa,* ***Crossed:*** *Tunica muscularis* ***Arrows:*** *iNOS positive cells*

In the third group, in the surface epithelial cell cytoplasm and stomach gland cells cytoplasm showed iNOS immunoreactivity. In the superficial mucous cell cytoplasm, isthmus and neck mucous cells, the iNOS immunoreactivity was mild, whereas the cytoplasm of the cells in the basal region was strongly stained (Figure 4).

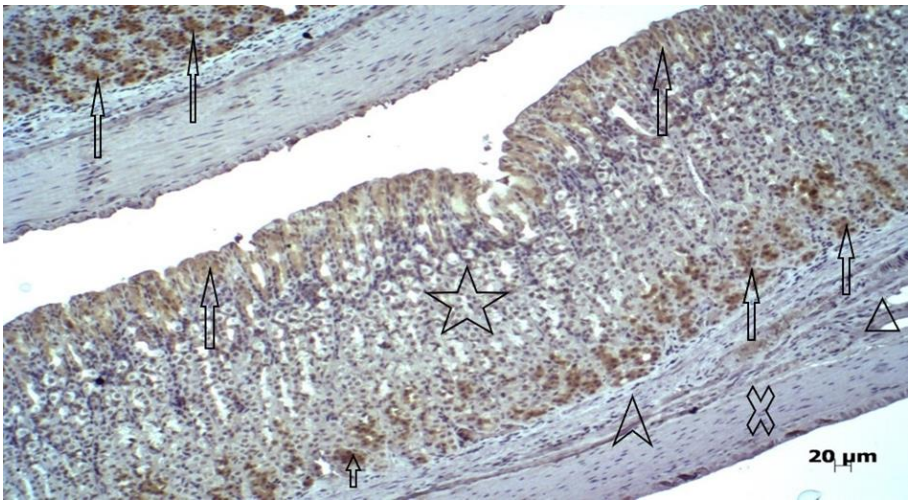

Figure 4. *For the third study group; 75% mussels + 25% standard rat diet was given every two days. Standard rat diet was given the other day. Rat stomach, (iNOSx10).* ***Star:*** *Lamina propria mucosa,* ***Pointed arrow:*** *Lamina muscularis mucosa,* ***Arrow head:*** *Lamina submucosa,* ***Crossed:*** *Tunica muscularis,* ***Arrow:*** *iNOS positive cells*

In the fourth group, in the surface epithelial cell cytoplasm and stomach gland cells cytoplasm showed iNOS immunoreactivity. In the superficial mucous cell cytoplasm, isthmus and neck mucous cells, the iNOS immunoreactivity was mild, whereas the cytoplasm of the cells in the basal region was strongly stained (Figure 5).

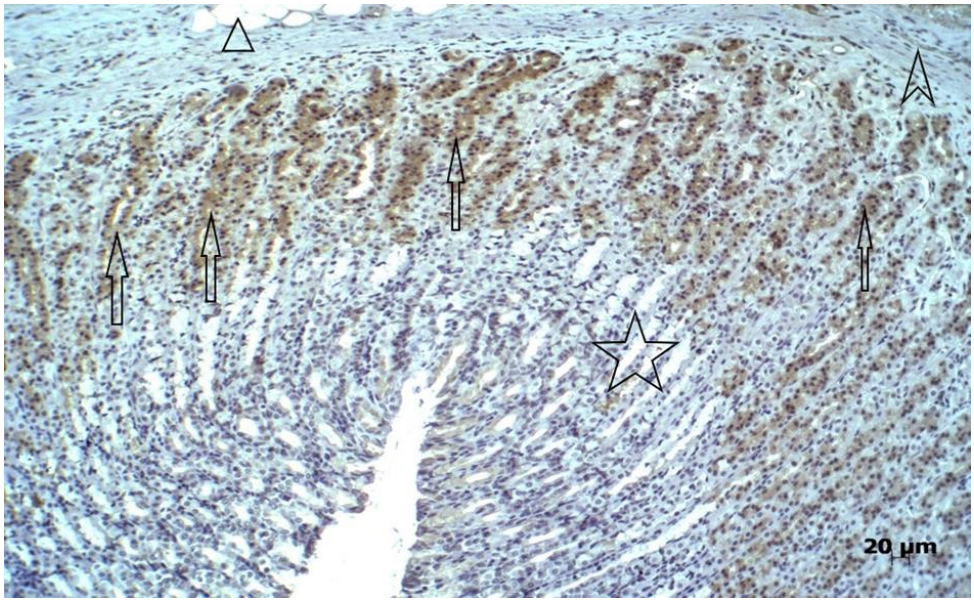

Figure 5. *For the fourth group; 75% mussels + 25% standard rat diet was given every three days. Standard rat diet was given the other two day. Rat stomach, (iNOSx10).* ***Star:*** *Lamina propria mucosa,* ***Pointed arrow:*** *Lamina muscularis mucosa,* ***Arrow head:*** *Lamina submucosa,* ***Arrows:*** *iNOS positive cells*

In the epithelial cells of the gastric mucosa in the all group, iNOS immunopositive cells could not be detected by negative staining (Figure 6).

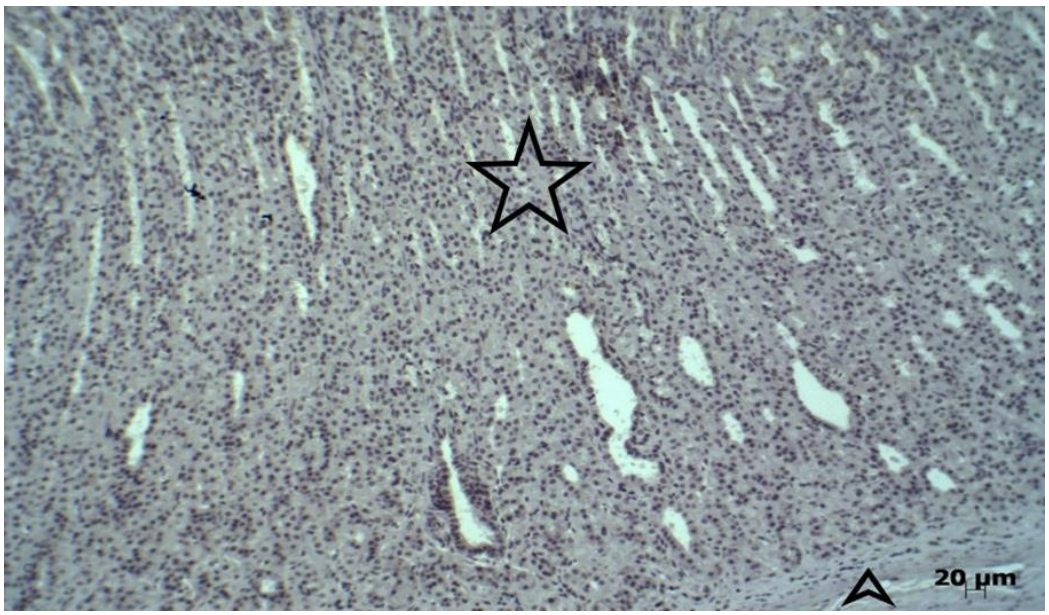

Figure 6. *For the second study group; 75% mussels + 25% standard rat diet standard rat feeds were given daily. Rat stomach, negative control, (iNOSx10).* ***Star:*** *Lamina propria mucosa,* ***Pointed arrow:*** *Lamina muscularis mucosa*

The increase of iNOS immunoreactivity in the gastric gland and surface epithelial cells of the stomach between the control group and the other groups was statistically significant. The iNOS immunoreactivity in the cytoplasm of mucus-secreting epithelial cells on the surface of the stomach and in the gastric gland cells of the lamina propria mucosa of the stomach was found 0% in the control group, 29.17% in the second group, 28.83% in the third group, 13.33% in the fourth group. There was

no meaning between the second and third groups. Statistical significance was determined between the second and third group with the fourth group (Figure 7).

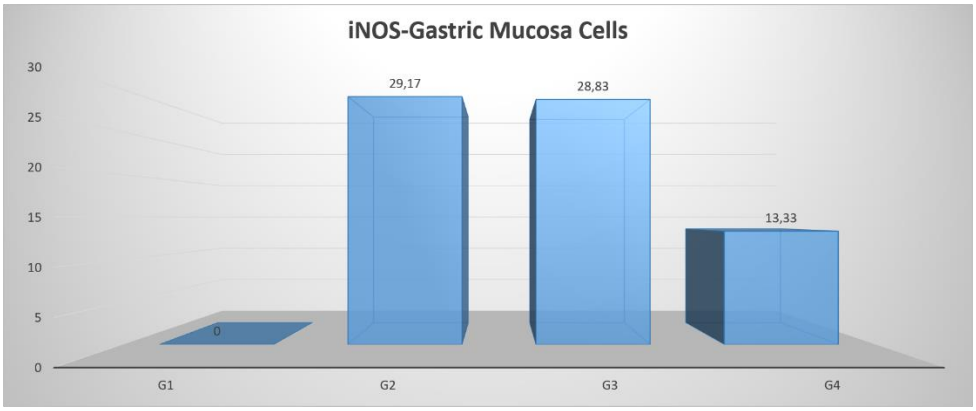

Figure 7. *iNOS immunoreactivity distribution between gastric mucosa cells*

The iNOS immunoreactivity in the cytoplasm of mucus-secreting epithelial cells of the stomach was found 0% in the control group, 1.50% in the second group, 1.50% in the third group and 1.50% in the fourth group. There was no meaning between the second, third and fourth groups (Figure 8).

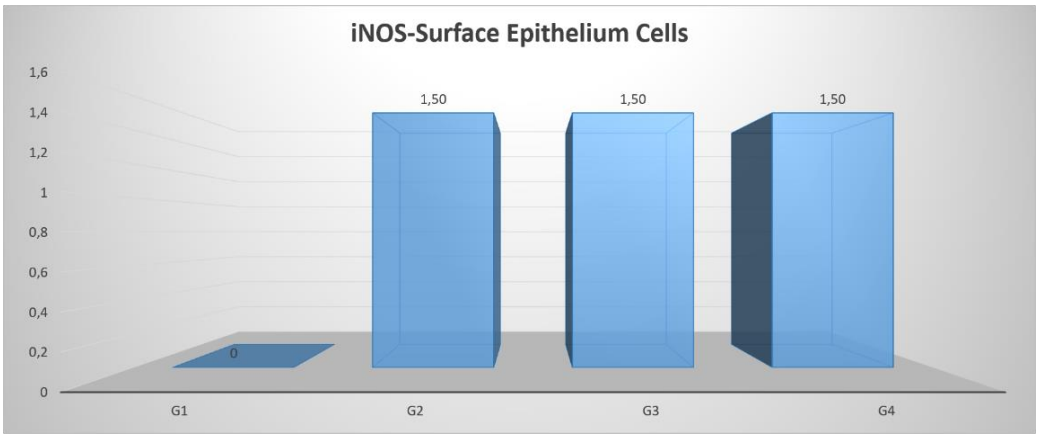

Figure 8. *Distribution of iNOS immunoreactivity in surface epithelium cells between groups*

The iNOS immunoreactivity increase was found to be statistically significant in the isthmus and neck region of the stomach between control group and other groups. The iNOS immunoreactivity in the cytoplasm of mucous neck cells and parietal cells in the neck region and the cytoplasm of stem cells and parietal cells in the isthmus region of the stomach was found 0% in the control group, 10.67% in the second group, 10.33% in the third group and 8.50% in the fourth group. There was no meaning between the second, third and fourth groups (Figure 9).

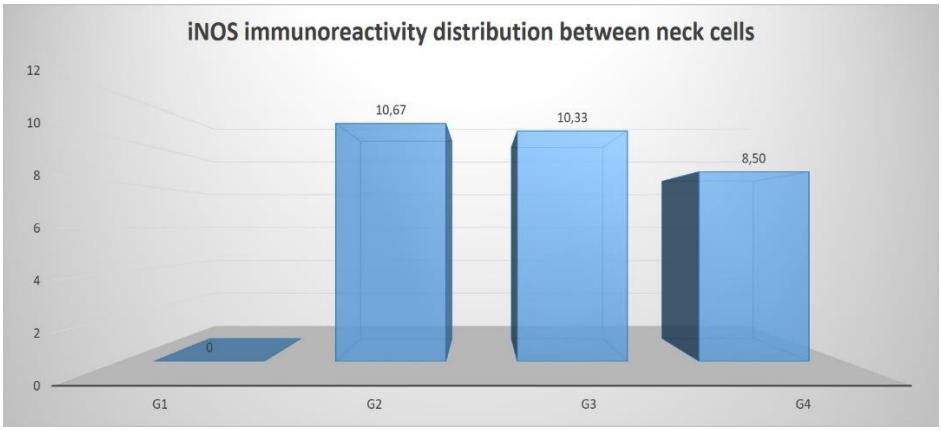

Figure 9: *iNOS immunoreactivity distribution between isthmus and neck regio cells*

The increase of iNOS immunoreactivity in the basal region cells of the stomach between the control group and the other groups was statistically significant. The iNOS immunoreactivity in the cytoplasm of the chief cells, endocrine cells and parietal cells in the basal region of the stomach was found 0% in the control group, 42.83% in the second group, 34.50% in the third group and 23.17% in the fourth group. Statistical significance was determined between the second and third group with the fourth group (Figure 10).

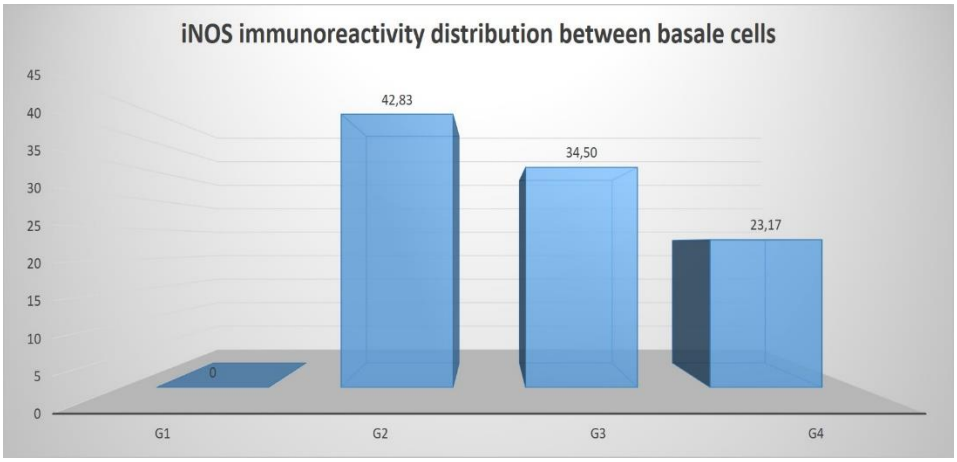

Figure 10. *iNOS immunoreactivity distribution between basale regio cells*

4. DISCUSSION

In this study the presented data showed that consumption of mussels caused an increase in iNOS in the gastric mucosa.

Our previous researches have shown that some mollusc species such as mussels grown in the Dardanelles, sea grass, sea chestnut, aquiva, and heavy metals such as iron, zinc, aluminum, lead and copper are found in sea water (Gezen et. al., 2011; Demir et. al. 2011; Gezen et al. 2011) [3-5]. Many research (Ibrahim et. al., 2000; Valko et. al., 2005; Gonçalves and Silva, 2007; Goyer, 1996; Ruff et. al., 1996; Aimo and Oteiza, 2006; Yokel et. al., 2008; Ittel, 1993) and official explanations (IARC, 1987) suggest that heavy metals damage the tissues of living things.

Gastric carcinogenesis (GC) is considered as a multistage progressive process. The early indicator for GC predisposition is abnormal hyperproliferation of gastric epithelial cells, such as chronic atrophic gastritis (CAG), dysplasia (DYS) and intestinal metaplasia (IM), which have been considered as

precancerous lesions of GC (Correa, 1992; You et. al., 1993). In a study by Feng et al., when the lesions progressed from normal to chronic superficial gastritis (CSG), CAG, IM, DYS, and finally to GC, the positive immunostaining rates for p53, iNOS, and VEGF were found to be significantly increased (Feng et al., 2002). The study demonstrated that the positive immunostaining rates of iNOS were correlated well with GC lymph node metastasis. All these findings suggested a role of NO in the initiation and progression of GC. NOS can also deaminate DNA and cause mutations of tumor suppressor genes, and possibly other oncogenes, such as c-met, and initiate genetic alterations of gastric cells leading to gastric malignancy (Wink et al., 1992). Nitric oxide (NO) is a free radical that is involved in the inflammatory process and carcinogenesis. iNOS is an inducible and key enzyme in the inflamed tissue. Recent literatures indicate that NO as well as iNOS and eNOS can modulate cancer-related events including nitro-oxidative stress, apoptosis, cell cycle, angio-genesis, invasion, and metastasis (Yang et al., 2009). Mononuclear inflammatory cells were found in the gastric mucosa of rats fed with mussel (Muratlı and Gezen 2018). $PbCl_2$ increases iNOS expression in an islet b cell line may be of pathological significance (Walther et al. 1999). In our previous study, we have detected very high levels of iNOS immunoreactivity in ovaries of female rats fed with mussels that we collected from the same region (Gezen et. al. 2018). In our study, we detected an increase in iNOS in the gastric mucosa. It therefore is conceivable that chronic exposure to heavy metal salt has a significant impact on gastric gland epithelial cells physiology and pathology.
In our study, iNOS release was detected in the gastric mucosa of rats consuming mussels. In addition to the findings of other researchers, we believe that heavy metals or other contaminants in sea water cause an increase in iNOS in the gastric mucosa.

Footnotes

*At the time of this research, she was working at Department of Pathology of Canakkale Onsekiz Mart University.

References

Aimo L., Oteiza PI., 2006. Zinc deficiency increases the susceptibility of human neuroblastoma cells to lead-induced activator protein-1 activation. Toxicol Sci. 91, 184–191.

Avunduk MC, Tavlı Ş, Yol S, Tavlı L, Yavuz A, Güngör S, Yılmaz O., 2000. Mide karsinomlarında hücre proliferasyon belirleyicisi olarak PCNA, Ki 67 ve agNOR kullanımı. Ankara Üniversitesi Tıp Fakültesi Mecmuası. 53(1), 11-15.

Bakır K., Kapucuoğlu N., Bülbül HD., 1996. PCNA (Proliferating Cell Nuclerar Antigene) immunohistokimyasal boyanması ve agNORs'un meme karsinomunda prognozu belirlemedeki rolü. Türk Patoloji Dergisi. 12(1), 35-38.

Chen L., Wang Y., Kairaitis LK., Zhang BH., Harris DC., 2001. Molecular mechanisms by which iron induces nitric oxide synthesis in cultured proximal tubule cells. Exp Nephrol. 9(3), 198-204.

Correa P., 1992. Human gastric carcinogenesis. A multistep and multifactorial process–first American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res. 52, 6735–6740.

Cui L., Takagi Y., Wasa M., Iiboshi Y., Khan J., Nezu R., Okada A., 1997. Induction of nitric oxide synthase in rat intestine by interleukin-1 alpha may explain diarrhea assciated with zinc deficiency. J Nutr. 127, 1729-1736.

Cuzzocrea S., Persichini T., Dugo L., Colasanti M., Musci G., 2003. Copper induces type II nitric oxide synthase in vitro. Free Radical Biol Med. 34(10), 1253-1262.

Demir N., Gezen MR., Ballı M., 2011. Çanakkale Boğazı'nın Karacaören Kıyısındaki Deniz suyu ve Bazı Yumuşakçalarda (Bivalvia ve Gastropoda) Ağır Metal Düzeylerinin Araştırılması. Ekoloji 2011 Sempozyumu. 5-7 Mayıs, Düzce, 177.

Eckhardt W., Bellmann K., Kolb H., 1999. Regulation of inducible nitric oxide synthase expression in b cells by environmental factors : heavy metals Biochem. J. 338, 695-700.

Feng CW., Wang LD., Jiao LH., Liu B., Zheng S., Xie XJ., 2002. Expression of p53, inducible nitric oxide synthase and vascular endothelial growth factor in gastric precancerous and cancerous lesions: correlation with clinical features. BMC Cancer. 2(8), 1–7.

Gezen MR., Demir N., Çetin M., 2011. Çanakkale Boğazı'nın Umurbey Kıyısındaki Deniz suyu ve Bazı Yumuşakçalarda (Bivalvia ve Gastropoda) Ağır Metal Düzeylerinin Araştırılması. Ekoloji 2011 Sempozyumu. 5-7 Mayıs, Düzce, 178.

Gezen MR., Demir N., Çetin M., Ballı M., 2011. Çanakkale Boğazı'ndaki Deniz Kestanesi (Paracentrotus lividus)' nin Ağır Metal Düzeyleri Üzerinde Bir Araştırma. Ekoloji 2011 Sempozyumu. 5-7 Mayıs, Düzce, 176.

Gezen MR., Muratlı A., Hacıvelioğlu SO., 2018. iNOS Immunoreactivity in the ovaries of rats feeding with mussel (*Mytilus galloprovincialis*). JSP. 2(2), 61-72.

Gonçalves PP., Silva VS., 2007. Does neurotransmission impairment accompany aluminium neurotoxicity? J Inorg Biochem. 101 (9), 1291–1338.

Goyer RA., 1996. Results of lead research: prenatal exposure and neurological consequences. Environ Health Perspect. 104 (10), 1050–1054.

Ibrahim D., Froberg B., Wolf A., Rusyniak DE., 2006. Heavy Metal Poisoning: Clinical Presentations and Pathophysiology, Clin Lab Med. 26 (1), 67–97.

International Agency for Research on Cancer, 1987. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Arsenic and Arsenic Compounds (Group 1), vol. 7, World Health Organization, Lyon, France, 100–106.

Ittel TH., 1993. Determinants of gastrointestinal absorption and distribution of aluminium in health and uraemia. Nephrol Dial Transplant. 8(1), 17–24.

Muratlı A., Gezen MR., 2018. Histopathological changes in the stomach tissue of rats fed with mussels (Mytilus galloprovincialis). 2. International Rating Academy Congress on Hope, 19-21 April 2018, 112.

Ni Z., Morcos S., Vaziri ND., 1997. Up-reulation of renal and vascular nitric oxide synthase in iron-deficiency anemia. Kidney Int. 52(1), 195-201.

Ruff HA., Markowitz ME., Bijur PE., Rosen JF., 1996. Relationships among blood lead levels, iron deficiency, and cognitive development in two-year-old children. Environ Health Perspect. 104, 180–185.

Tian L and Lawrence DA., 1996. Metal-induced modulation of nitric oxide productionin vitro by murine macrophages: lead, nickel, and cobalt utilize different mechanisms. Toxicol Appl Pharmacol. 141(2), 540-547.

Valko M., Morris H., Cronin MT., 2005. "Metals, toxicity and oxidative stress," Current Medicinal Chemistry. 12 (10), 1161–1208.

Wink DA., Kasprzak KS., Maragos CM., Elespuru RK., Misra M., Dunams TM., Cebula TA., Koch WH., Andrews AW., Allen JS., 1992. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. Science. 254, 1001–1003.

Yang GY., Taboada S., Liao J., 2009. Induced nitric oxide synthase as a major player in the oncogenic transformation of inflamed tissue. Methods Mol Biol. 512, 119-56.

Yokel RA., Hicks CL., Florence RL., 2008. Aluminum bioavailability from basic sodium aluminum phosphate, an approved food additive emulsifying agent, incorporated in cheese. Food Chem Toxicol 46, 2261–2266.

You WC., Blot WJ., Li JY., Chang YS., Jin ML., Kneller R., Zhang L., Han ZX., Zeng XR., Liu WD., Zhao L., Correa P., Fraumeni JF., Xu GW., 1993. Precancerous gastric lesions in a population at high risk of stomach cancer. Cancer Res. 53, 1317–1321.