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Histopathological Changes in The Spinal Cord Tissue of Rats Administered an Experimental Mussel Diet

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Abstract

Aim: Regional eating habits show that it causes neurodegenerative problems due to heavy metals that can accumulate in consumed foods and affect tissues such as the nervous system. Since crustaceans such as mussels feed by filtering the water, they are exposed to toxic plankton and various chemicals, especially heavy metals. Due to the limitations of experimental studies on this subject, the effects of mussel consumption on the spinal cord were investigated.

Methods: In this study, histopathological changes in the spinal cord tissue of rats fed with shellfish collected from the Dardanelles were determined. The subjects were divided into two groups, and the first group was fed standard rat food for 4 weeks, and the second group was fed a mussel diet. At the end of the study, spinal cord tissue samples taken from rats were subjected to routine histopathological procedures and evaluated under a light microscope.

Results: In the experimental group, a decrease in the number of neurons in the medulla spinalis and an increase in the number of astrocytes were noted. TUNEL staining showed that apoptosis occurred intensively in glial cells, but did not occur in anterior and posterior horn motor neurons.

Conclusion: The findings showed that long-term mussel consumption can cause axonal damage in motor and sensory neurons and degeneration in glial cells. For this reason, it is important for health that marine diets in coastal areas are made with healthy and hygienic products.

Keywords: Medulla spinalis, neuronal degeneration, apoptosis, mussel

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Deneysel Midye Diyeti Uygulanan Sıçanların Omurilik Dokusunda Meydana Gelen Histopatolojik Değişiklikler

Öz

Amaç: Bölgesel beslenme alışkanlıkları, tüketilen besinlerde birikebilen ve sinir sistemi gibi dokuları etkileyebilen ağır metaller nedeniyle sinir sisteminde nörodejeneratif sorunlara yol açtığını göstermektedir. Midye gibi kabuklular suyu süzerek beslendikleri için başta ağır metaller olmak üzere zehirli plankton ve çeşitli kimyasallara maruz kalırlar. Bu konuyla ilgili deneysel çalışmaların kısıtlılığı nedeniyle midye tüketiminin omurilik üzerindeki etkileri araştırıldı.

Yöntemler: Bu çalışmada Çanakkale Boğazı'ndan toplanan kabuklu deniz ürünleri ile beslenen sıçanların omurilik dokusundaki histopatolojik değişiklikler incelendi. Araştırmada denekler iki gruba ayrılarak, birinci gruba 4 hafta boyunca standart sıçan yemi, ikinci gruba ise 4 hafta midye diyeti uygulandı. Çalışmanın sonunda sıçanlardan alınan omurilik dokusu örnekleri rutin histopatolojik işlemlere tabi tutularak ışık mikroskobunda değerlendirildi.

Bulgular: Deney grubunda medulla spinalisde nöron sayısında azalma, astrosit sayısında ise artış kaydedildi. TUNEL boyaması apoptozun glial hücrelerde yoğun olarak meydana geldiğini ancak ön ve arka boynuz motor nöronlarında oluşmadığını gösterdi.

Sonuç: Bulgular, uzun süreli midye tüketiminin motor ve duyu nöronlarında aksonal hasara ve glial hücrelerde dejenerasyona neden olabileceğini gösterdi. Bu nedenle kıyı bölgelerinde deniz diyetlerinin sağlıklı ve hijyenik ürünlerle yapılması sağlık açısından önemlidir.

Anahtar kelimeler: Medulla spinalis, nöronal dejenerasyon, apoptoz, midye, TUNEL.

INTRODUCTION

Neurotoxicity of metals is generally studied on a single metal basis. However, the reality is that humans are often exposed to an environment composed of mixed metals[1]. Various metal mixtures at different concentrations can affect body homeostasis. Metals Pb, As, Cd, Hg have been examined in animal models as mixtures and their effects on toxicity levels and various mechanisms that may lead to neurological disorders have been studied[2]. Metals are not biodegradable: therefore, organisms can accumulate them in an insoluble form for excretion from the organism or detoxify them by binding to "accessory" proteins[3]. Effects of heavy metals on the human body at the cellular level; oxidative stress, lipid peroxidation, mitochondrial dysfunction, apoptosis, DNA damage, disruption of ATP synthesis, DNA methylation, thiamine deficiency, decrease in acetylcholinesterase activity, formation of free radicals and reactive oxygen species, epigenetic changes, regulation of hypoxia inducible factor-1a and DNA It induces toxicity through pathways such as disruption of the repair system[4].

Heavy metals have an indisputable role in oxidative stress and harmful effects on the CNS. Since the brain is an organ that consumes large amounts of oxygen, it has a high potential for the production of free radicals and reactive oxygen species. Reactive oxygen species play an important role in neurodegeneration by targeting various biomolecules such as DNA, RNA, lipids and proteins in the nerve cell, and a wide range of processes such as nucleic acid oxidation and lipid peroxidation, and by changing the function of biomolecules[5]. Various cell types in the central nervous system (CNS) are affected by acute/chronic heavy metal exposure, and the functions and activities of intracellular organelles are impaired. This significantly affects the survival, proliferation, metabolism and other functions of CNS cells and leads to the onset of various age-related neurodevelopmental or neurodegenerative disorders. Heavy metal exposure not only damages the CNS but also affects the peripheral nervous system. It is reported that exposure to many heavy metals results in significant deterioration in peripheral nerves[6].

Technological developments and the innovations they brought with them enabled the acceleration of industrialization and existing production activities increased, the population became crowded, and the destruction and destruction of natural areas accelerated. In parallel with all these developments, aquatic environments have also had their share and are rapidly becoming polluted. Water pollution affects all its physical, chemical and biological properties. As a result, aquatic life is threatened and irreversible damage is caused to aquatic creatures. Drugs used in agriculture for food production, especially heavy metals, accumulate in aquatic creatures that manage to survive, even by chance, and can have negative effects on other creatures, especially humans, that feed on these creatures. The rapid increase in the world population has increased the need for food and the tendency towards alternative and delicious products. In this context, shelled aquatic creatures have taken their place in menus as a very popular food source. Especially in coastal areas, the consumption of creatures such as mussels, scallops and aquifers is considerable[7,8]. Our study was planned on the food chain that occurs with environmental pollution and can also affect humans. What kind of changes and damages can occur in our nervous system as a result of the consumption of shellfish, which live in water and can accumulate highly toxic substances such as heavy metals, as a result of environmental disasters such as water and air pollution, which increase day by day? We carried out the experimental study by searching for the answer to the question.

METHODS

Animal Model

In our study, 24 male Wistar albino rats (weighing 270-310 ± 10 g) were used. All rats were housed in an environment with an average temperature of 22 ± 1 °C, humidity of 55 ± 5, and a ventilation and air conditioning system with 12 hours of light and 12 hours of darkness. The rats were given as much water as they could drink. In feeding planning, standard rat food and mussels were given at 15% of each rat's weight. The scallops given as food to the rats were collected from designated locations in the Dardanelles. After the scallops were overcooked, the shell was broken and dried in an oven at 100 °C, turned into pellet feed and given to the subjects.

Control group (G1, n:6); The group given normal rat food;

Experiment (G2, n:6); The group was given 75% mussel + 25% standard rat food every day without interruption. Subjects in each group were fed for 30 days.

Histochemical Staining

The removed spinal cord samples were fixed in 10% formalin solution at room temperature for 48 hours and then passed through a series of increasing amounts of alcohol. Tissues were embedded in paraffin after being subjected to three changes of xylol for transparency. Hematoxylin-Eosin and TUNEL staining was performed by taking 3-5 micron thick sections from the blocked tissue samples.

TUNEL assay

Terminal deoxynucleotidyl transferarasiled UTP nick end labeling (TUNEL) method detects fragmentation in the DNA nucleus, was used in situ during apoptotic cell death, the apoptosis detection kit (ApopTag® Plus Peroxidase In Situ Apoptosis Kit). TUNEL staining were scored

apoptotic index (AI) (1000 cells were evaluated for the presence of apoptotic cells and apoptotic bodies. The AI was expressed as a percentage of the total number of non-apoptotic cells counted. One Way-ANOVA was compared with Tukey statistical test and $p<0.05$ results were considered statistically significant[9].

RESULTS

The histolopathological changes were not found in the spinal cord tissues of the control group subjects. In the spinal cord histological structure; A histological structure consisting of central canal, gray matter and white matter was observed. In the cortex, it was observed that the euchromatic structure of motor neurons was

accompanied by Nissl granules in their cytoplasm. Myelinated nerve fibers and neuroglia were seen in the medulla. In the H&E findings of the spinal cord tissues of the rats of the experimental group; Inflammation near the central canal, damage and decrease in number of neurons in the anterior and posterior horns of the cortex, nerve fiber damage, increase in neuroglia (gliosis) and vascular congestion were detected. Nissil granules were pushed to the periphery and a morphology with hyperchromatic and non-central pyknotic nuclei was observed in the motor neuron body. Degeneration of sensory neurons and dysregulation of glial cells were detected. In this study, we observed that glial cell-based damage in the spinal cord tissue of rats fed with mussels contaminated with heavy metals and other polluting factors also triggered degeneration in motor and sensory neurons (Figure 1,2).

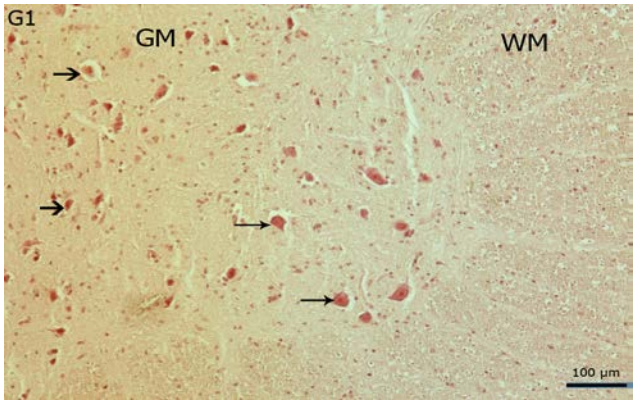

Figure 1. G1: Control group medulla spinalis histological structure, HE, scale bar: 100 µm (short arrow: sensory neuron, long arrow: motor neuron, GM: gray matter, WM: White matter).

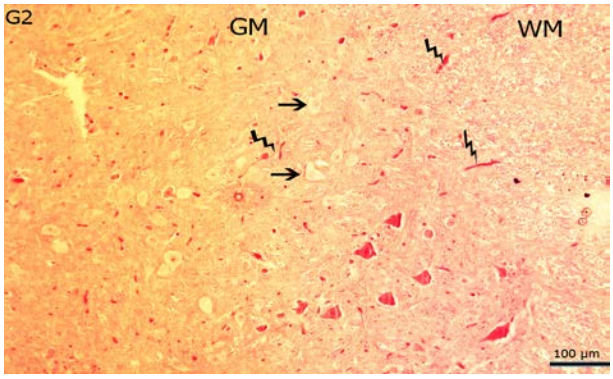

Figure 2. G2: Medulla spinalis tissue of the group given mussels, H.E, scale bar: 100 µm (lightning bolt: congestion, arrow: degenerated sensory neuron).

TUNEL Assay findings

TUNEL staining was performed to determine apoptosis. No motor or sensory neurons undergoing apoptosis were found in the control group G1 spinal cord tissues. We observed that a very small amount of apoptosis occurred in neuroglia cells. In the spinal cord tissues of the subjects given mussels, it was determined that severe apoptosis occurred in neuroglia and that there was a weak apoptosis activity in the nuclei of motor and sensory neurons. This picture suggests that neuronal damage may be triggered when glial damage occurs for a long time at the cellular level. Findings were obtained proving that neuronal damage may increase when feeding time due to mussel consumption is increased (Figure 3, 4, 5, 6). In the semiquantitative evaluation made considering the severity of apoptosis, a statistically significant difference was detected between the control group and the experimental group (Figure 7).

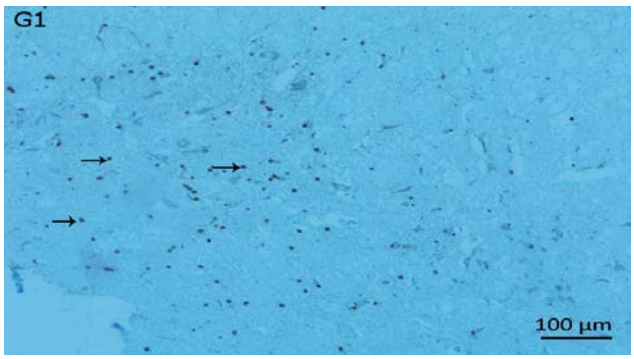

Figure 3. G1: Control group medulla spinalis tissue TUNEL staining, scale bar: 100 µm (arrow: apoptotic cells).

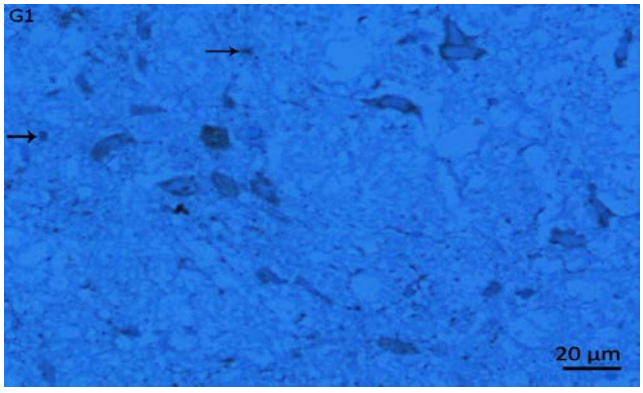

Figure 4. G1: Control group medulla spinalis tissue TUNEL staining, scale bar: 20 µm (arrow: apoptotic cells).

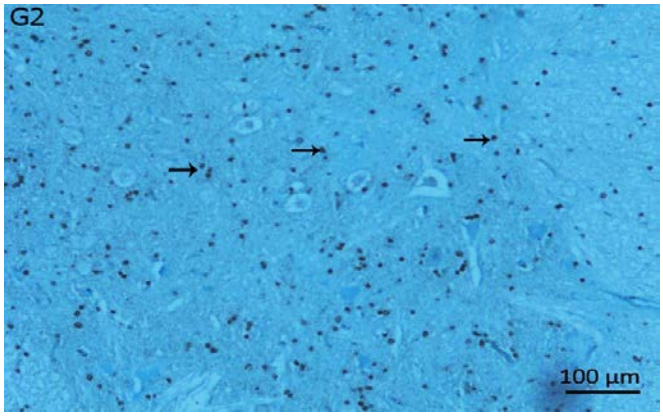

Figure 5. G2: TUNEL staining of medulla spinalis tissue of the group given mussels, scale bar: 100 µm(arrow: apoptotic cells).

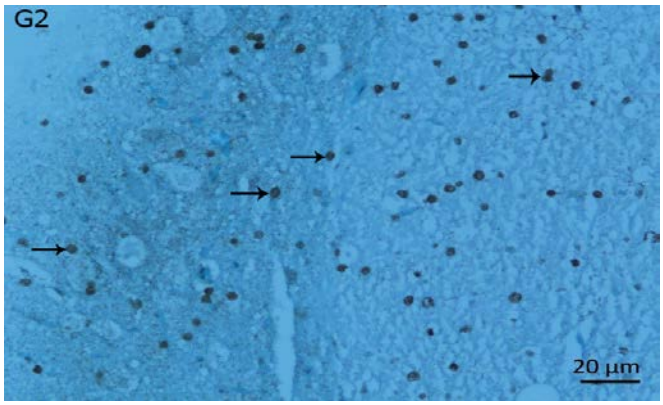

Figure 6. G2: TUNEL staining of medulla spinalis tissue of the group given mussels, scale bar: 20 µm (arrow: apoptotic cells).

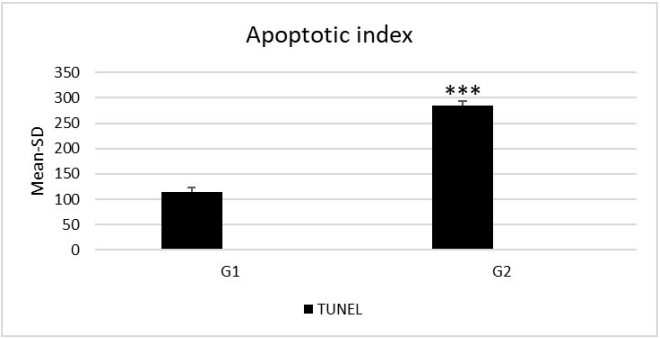

Figure 7. Apoptotic index in spinal cord of rats (G1: Control group, G2: Experimentally group).

Table I: Heavy metal concentrations of mussel muscle tissue (µg/g dry weight.)

Heavymetals Region	*Cd*	*Pb*	*Cu*	*Zn*
Çamburnu	1.24	0,62	1.54	21.24
Yenikordon	1.38	0,72	1.66	19.84
Lapseki	1.21	0,54	0.94	18.35
Mean	1.27	0.62	1.38	19.81

DISCUSSION

In many studies, it has been stated that the accumulation of heavy metals in the sea disrupts the ecological balance and that these substances, which can accumulate in seafood, which constitute an important part of the food chain, cannot be ignored as they can cause serious permanent health problems, especially to children, through the food chain[10]. Geographically, the Dardanelles is one of the regions that receives its share of this pollution due to transit ship passages. In the research conducted on sea water and some shellfish growing in this region, it was reported that the accumulation of elements such as Zn, Mn, Al, Fe and Cu in the internal organs of these creatures was above acceptable levels[11,12].

Metals differ from other toxic substances in that they cannot be produced or destroyed by the human body[13]. Mercury, lead, cadmium, arsenic and nickel are reported as the most dangerous heavy metals. Heavy metals can displace basic metals from their binding sites in proteins and enzymes. Furthermore, a disturbance in metal homeostasis often affects the distribution of another metal and can lead to disturbances in enzymatic pathways; toxic metals can interact with nuclear proteins and DNA, causing site-specific damage and production of reactive oxygen species (ROS). Metals are not biodegradable: therefore organisms can accumulate them in an insoluble form for excretion from the organism or detoxify them by binding to "accessory" proteins[3]. The findings obtained in our study showed that heavy metal accumulation can be transferred from living to living through food (Table 1).

It is known that spinal cord injuries mostly occur due to degeneration of anterior motor neurons and posterior sensory neurons in the cortex. The neurotoxic effect of heavy metals, especially heavy metals such as cadmium and lead, has been proven by many scientific studies. Glial and neuronal changes have been

described during development following chronic or acute Pb poisoning, but little is known about the effects of chronic lead poisoning in the spinal cord in adults. However, there is evidence in the zebrafish model system that Pb causes loss of motor-neuron extension and apoptosis in the spinal cord[14] and this neuronal dysfunction has been reported to result from damage to glial cells that support neurons[15]. Degeneration of sensory neurons and glial cell damage increased in the spinal cord tissues of rats fed mussels. Accordingly, it was shown that the apoptotic mechanism was also triggered and toxicity may increase depending on time.

Various cell types in the CNS are affected by acute/chronic heavy metal exposure, and the functions and activities of intracellular organelles are impaired. This significantly affects the survival, proliferation, metabolism and other functions of CNS cells and leads to the onset of various age-related neurodevelopmental or neurodegenerative disorders[4]. In a study, it was found that heavy metals were found in many spinal interneurons and a few α motor neurons in most of the elderly people who participated in the study, and that toxic metals in the future could damage inhibitory interneurons and cause excitotoxic damage in motor neurons and cause amniotrophic lateral sclerosis, multiple sclerosis (MS), sarcopenia. It is predicted that the underlying cause of motor neuron damage or loss in cases such as calf fasciculations and calf fasciculations may be heavy metal exposure[16]. Heavy metal exposure not only damages the CNS but also affects the peripheral nervous system. It has been reported that co-exposure to many heavy metals results in significant impairment of peripheral nerves[6]. We found differences in neurons and neuroglial morphology between the control group and the feeding mussel group. It has been reported that after the 90 days of exposure of young adult rats to Pb in drinking water, the integrity of the myelin sheath in brain is affected by revealing disintegration of its multi-lamellar structure, these results correlate with what we found in the altered morphology of glial cells in the present study[17]. In a study conducted on rats, it was reported that cadmium application significantly reduced retention of information in memory, decreased acetylcholine levels and high acetylcholinesterase activity were observed in the frontal cortex and hippocampus of rats treated with cadmium, and a significant increase in malondialdehyde levels occurred after cadmium application[18].

Apoptosis was more important in the glia cells for example oligodendrocyte of the mussel feeding group than in the neuronal cells of this group. This realty permit of to qualify that in the neurodegenerative process, the damage is not particular to the neuron, but it is also a convergence of damage in all glial cells. In this case, glial cells are the primarily affected cells in the neurodegenerative process induced by mussel, showing a total chromatin fragmentation in white matter in contrast with gray matter.

In spinal cord histopathology, loss of neurons, edema, vacuole formation, deformation of Nissl granules and gliosis were detected[19]. It has been reported that in the spinal cord of rats fed with lead added to their drinking water for three months, degeneration occurred severely in oligodendrocytes, the myelin sheath became thinner, and the number of neurons decreased, but the number of astrocytes increased[20]. Our findings are obtained from the studies conducted; It supports the findings of neurotoxicity that may occur both as a result of irregular use of drugs and as a result of exposure of products such as fish and shellfish obtained from seas and lakes to herbicides and heavy metals. Significant sensory neuron damage was observed in the experimental group of our study. In one of the studies, it was

reported that myelin formation was prevented as a result of damage to oligodendrocytes as a result of lead exposure. It has also been reported that gliosis and excessive microglial response occur as a result of apoptosis[21,22].

CONCLUSION

When studies on heavy metal exposure are examined, it is seen that acute and high dose exposure causes toxic effects; It is observed that chronic and low dose exposure causes heavy metal accumulation in tissues over time. Healthy foods consumed are also important for the nervous system. Toxic substances, especially those that can be transported through food, can accumulate in cells over time and cause permanent damage. It is important that marine products are sourced from reliable sources and are hygienic. There is a need to investigate experimental CNS and peripheral system diseases regarding the transport of heavy metals and other toxic substances through food.

Ethics Committee Approval: Ethical protocol was approved by Çanakkale Onsekiz Mart University Ethics Committee, approval number (Decision No: 2020/04-07).

Conflict of Interest: The authors declarednoconflicts of interest.

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