© by PSP Volume 30– No. 02/2021 pages 1240-1248 Fresenius Environmental Bulletin

INVESTIGATION OF SPINAL CORD DAMAGE IN RATS FED WITH CLAM (*TAPES DECUSSATUS)* BY TUNEL ASSAY

Latife Ceyda Irkin*

Canakkale Onsekiz Mart University, Canakkale Faculty of Applied Science, Department of Fisheries Technology, Canakkale, TURKEY

ABSTRACT

Toxicological and epidemiological evidence suggests that heavy metals cause neurodegenerative problems. Since crustacean are fed by filtering seawater, they can be exposed to factors such as heavy metals, pesticides, herbicides, toxic plankton, and household waste. It has been determined that aluminum and lead, which are metal groups with high neurotoxicity in humans, are particularly high in crustaceans. We found that experimental studies related to this subject were very limited and we did not find any studies on the histopathological effects of bivalve consumption on the medulla spinalis tissue. This study was conducted to determine cellular damage and apoptosis in the spinal cord tissue of rats fed with clams collected from determined locations in the Dardanelles Strait. The study was divided into four groups, the first group was the control group (n = 6) fed standard rat feed for three weeks, the second group (n = 6) 80% clams and 20% standard feed every day, the third group 80% clams every two days and 20% standard feed, the fourth group was determined as the experimental group to which 80% clams and 20% standard feed were applied every three days. At the end of the study, spinal cord tissue samples taken from rats were evaluated under a light microscope after routine histopathological procedures. While there is a decrease in the number of neurons in the experimental group, the increase in the number of astrocytes draws attention. TUNEL staining showed that apoptosis occurred intensely in glial cells, and mild apoptosis occurred in anterior and posterior horn motor and sensory neurons. As a result, it shows that long-term consumption of shellfish causes axonal damage of motor and sensory neurons and degeneration of glial cells. It is known that damage to glial cells will trigger neuronal damage. It is very important in terms of health that we feed ourselves with healthy and hygienically grown products.

KEYWORD:
Apoptozis, TUNEL assay, clam, spinal cord, rat

INTRODUCTION

The rapid development in technology causes the increase of human population and their needs. This has led to the common use of various chemical agents to increase efficiency in agriculture and industry. Heavy metals and herbicides are chemicals used to increase the product quality and agricultural yields; many of these chemicals can easily mix with rain and wastewater and flow into lakes and seas, which can lead to accumulation of undesirable chemical agents in fish and aquatic organisms [1]. The contamination of the ecosystem with chemicals indicates that the demand in fish and seafood will increase its orientation towards products produced under more hygienic conditions.

Clams are a good source of nutrients and consumed by people in the form of stuffed and fried. While crustacean accumulate heavy metals by filtering the sea water, they can also face to other factors such as toxic plankton, pesticides, and domestic waste. Mollusks (mussel, sea snail, oyster, etc.) grown in different parts of the Dardanelles in the autumn (Zn, Al, Fe). Heavy metals can change the chemical structure of water accumulated by the tissues of mussel [2]. Mussels are very sensitive to the chemical structure of water [3]. Conducting research examining the accumulation and damage of heavy metals in aquatic organisms is important in terms of identifying biochemical, physiological, structural, and functional disorders that may occur in the organism as well as determining the species with high sensitivity to these metals. Metallic pollutants measured as an indicator of environmental pollution that can often reach high levels, especially in seafood. In this way, metal residues such as mercury, cadmium and lead, and pesticides at low levels but continuously taken with nutrients, significantly affect the environment and human health [4].

Heavy metal is defined as metals with all toxic properties regardless of the atomic weights of the elements. Although more than sixty elements can be given as examples of heavy metals, the most common and most recognized elements are mercury (Hg), aluminum (Al) manganese (Mn), iron (Fe), lead (Pb) are some of the heavy metals [5, 6]. The main reason of the toxic effect of heavy metals on the body is the disorders that they create in the intra-

cellular metabolic processes. DNA damage is autoimmune and neurological diseases with mitochondrial damage and induction of apoptosis. The treatment possibilities of these problems caused by heavy metals are limited and death can be observed frequently. Mercury, lead, cadmium, and copper are among the heavy metals with the toxic effects [4, 7]. Toxic heavy metals damage the nerves and bones, block the functions of important enzyme groups and cause cancer. In recent studies, it is thought that the most important cause of degenerative diseases such as Alzheimer's and Parkinson's diseases may be heavy metals accumulation [8]. Pb has been reported to play an important role in diseases such as amyotrophic lateral sclerosis at high concentrations [9].

It is known that medulla spinalis injuries mostly occur due to the degeneration of anterior motor neurons and posterior sensory neurons in the cortex. The neurotoxic effect of heavy metals, especially cadmium and lead, has been proven by many scientific studies. Glial and neuronal changes have been identified during the development following chronic or acute Pb poisoning. Lead has been suggested to damage motor neurons after retrograding axonal transport from the neuromuscular junction [10], which is related to the fact that motor neurons are the most vulnerable cell type in neurodegenerative diseases. In other hands there is evidence that Pb transported from the muscle does not damage spinal motor neurons [11]. However, in the zebrafish model, there is evidence of Pb's loss of motor - neuron extension and apoptosis in the spinal cord [12], and this neuronal disfunction has been reported to be caused by damage the glial cells that support neurons [13]. In diets based on crustacean consumption such as mussels with high nutritional value, the collection of heavy metals makes the neurotoxic effects. In mollusks grown in different parts of the Dardanelles, some heavy metals (Zn, Al, Fe, Pb) were found to be above for acceptable values based on these results.

MATERIALS AND METHODS

Animal Model. The rats were kept under optimum conditions for three weeks in a 12-hour light cycle with sufficient water and feeding. Randomly rats were divided into the two groups. The control group (n: 6); A diet with standard rat food was applied daily. For the second experimental group (n: 6), a diet prepared with 80% clam + 20% standard rat food was applied Daily again. Rats were fed twice a day for three weeks, with the amount of prepared feed corresponding to 15% of their weight at the same time every morning and evening. Clams that were given to rats as food were collected from Camburnu region of Canakkale Strait in April and May 2020. After the beaks were cooked, the crust was broken and dried at 100°C. The dried meat was converted into rat feed form and given to the rats.

Ethics Statement. A total of 24 male Wistar albino rats were used in the study. The study protocol was approved by the Canakkale Onsekiz Mart University Ethics Committee for Animal Research (Protocol number: 2020/04-06).

Heavy Metal Analysis. Heavy metal analysis of muscle tissues taken from clam samples collected in the spring season was performed at the Central Laboratory of Canakkale Onsekiz Mart University with the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Perkin Elmer, Optima 8000) device. Cadmium (Cd), lead (Pb), copper (Cu), iron (Fe) and zinc (Zn) heavy metal results were calculated as mg kg^{-1} wet weight [14].

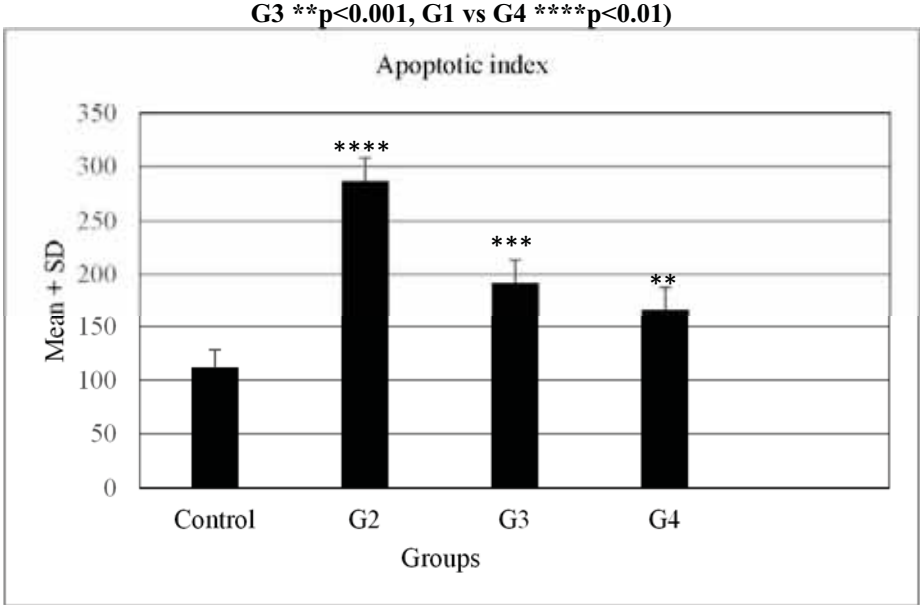
Histochemical Staining. The animal model of our study was for four weeks. At the end of the period, the spinal tissues of the rats, which were then anesthetized with rompun and xylazine, were removed and placed in tissue transport cassettes and fixed for 24 hours in immunofix. Afterwards, tissue samples are passed through graduated alcohol solutions and purified from the water in the tissue; Alcohol in tissues was cleaned with xylen, tissue samples were kept in paraffin in the oven and then blocked-in base mode. Blocked tissue samples were cut 3-5 microns thick with microtome and placed on slide and placed in preparation boxes. Hematoxylin-Eosin and Toluidine blue staining were applied to histochemical dyes on tissue samples.

TUNEL assay. Terminal deoxynucleotide transferarasile dUTP 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). All reagents listed below and the manufacturer's instructions below. Five-millimeter thickness testis sections were deparaffinized in xylene and rehydrated with an ethanol series rated as previously described. They were then incubated for 20 minutes with 20 mg/mL of proteinase K and rinsed in tris-buffered saline. Endogenous peroxidase activity was inhibited by incubation 3% hydrogen peroxide. The sections were then incubated for 10 min to 30 min with the equilibration buffer, then the TdT enzyme in a humidified atmosphere at 37°C for 90 min. Then, pre-warmed working power for 10 minutes at room temperature was placed in stop/wash buffer and incubated with blocking buffer for 30 minutes. Each step was separated by extensive washings in tris-buffered saline. Labeling was elicited using diaminobenzidine tetrahydrochloride, contrast staining was carried out using methyl green and the sections were dehydrated, cleared, and mounted [15]. TUNEL staining were scored semi-quantitatively to determine the number of positive staining, none (-), weak (+), moderate (++), high (+++), very high (++++). These analyses were

TABLE 1
Heavy metal concentrations of *Tapes decussatus* muscle tissue (µg/g dry weight)

Heavy metals Region	Cd	Pb	Cu	Zn
Camburnu	1.30	0,68	1.47	20.74
Yenikordon	1.18	0,60	1.24	18.86
Cardak	0.94	0,45	0.82	18.24
Average value	1.14	0.77	1.17	19.28

TABLE 2
Apoptotic reactivity distribution of groups in spinal cord of rats (Control (G1) vs G2 ****p<0.0001, G1 vs G3 **p<0.001, G1 vs G4 ****p<0.01)



performed in 2 sections for each animal, at a rate of 40X magnification for at least 10 different regions per section.

Evaluation of tissue samples and statistics. All stained tissue samples were evaluated by Zeiss AXIO Scope 1 brand research microscope and photographed with digital camera (AxioCam ICc 3). TUNEL positive cells were detected using the Leica LAS V3.8 image analysis system. Apoptotic index was evaluated by examining with H-score. The staining rate was rated semi quantitatively; 0, if less than 1% of cells are stained; if 1-10% of cells are stained 1+; if 11-50% of cells are stained, 2+; if 51-80% of cells are stained 3+; if more than 80% of cells were stained, it was evaluated as 4+. Also, the intensity of staining 0 = no staining; 1 = pale; 2 = moderate; 3 = determined intensely by blind method. Then the total score was calculated with the formula ((1 + staining intensity/3) x staining rate). The result data were compared with the One Way-ANOVA, Tukey statistical test and $p < 0.05$ results were considered statistically significant [16].

RESULTS

H&E Results. Rats determined as control group were given standard rat feed and when the spinal tissues were evaluated histologically, no impaired structure was encountered. Histological structure consisting of central channel, gray matter and white matter was dominant in the control group. In the gray matter, motor neurons were found to exhibit a heterometric body with a chromatic nucleus, prominent nucleolus, Nissl granules in the cytoplasm and a heterogeneous body. The white matter consists of myelinated nerve fibrils and a small number of neuroglia cells. In H&E staining of the spinal cords of clam-fed rats, inflammation around the central canal, damage in nerve fibrils, damage to neuroglia (oligodendrocyte, astrocyte, microglia) cells (gliosis) and vascular congestion were detected. In motor neurons, the periphery of Nissl granules is pushed and empty spaces form, with hyperchromatic and noncentral pycnotic core morphology. In sensory neurons were showed little body structures and irregular distribution of glia cells (Figure 1 and Figure 2).

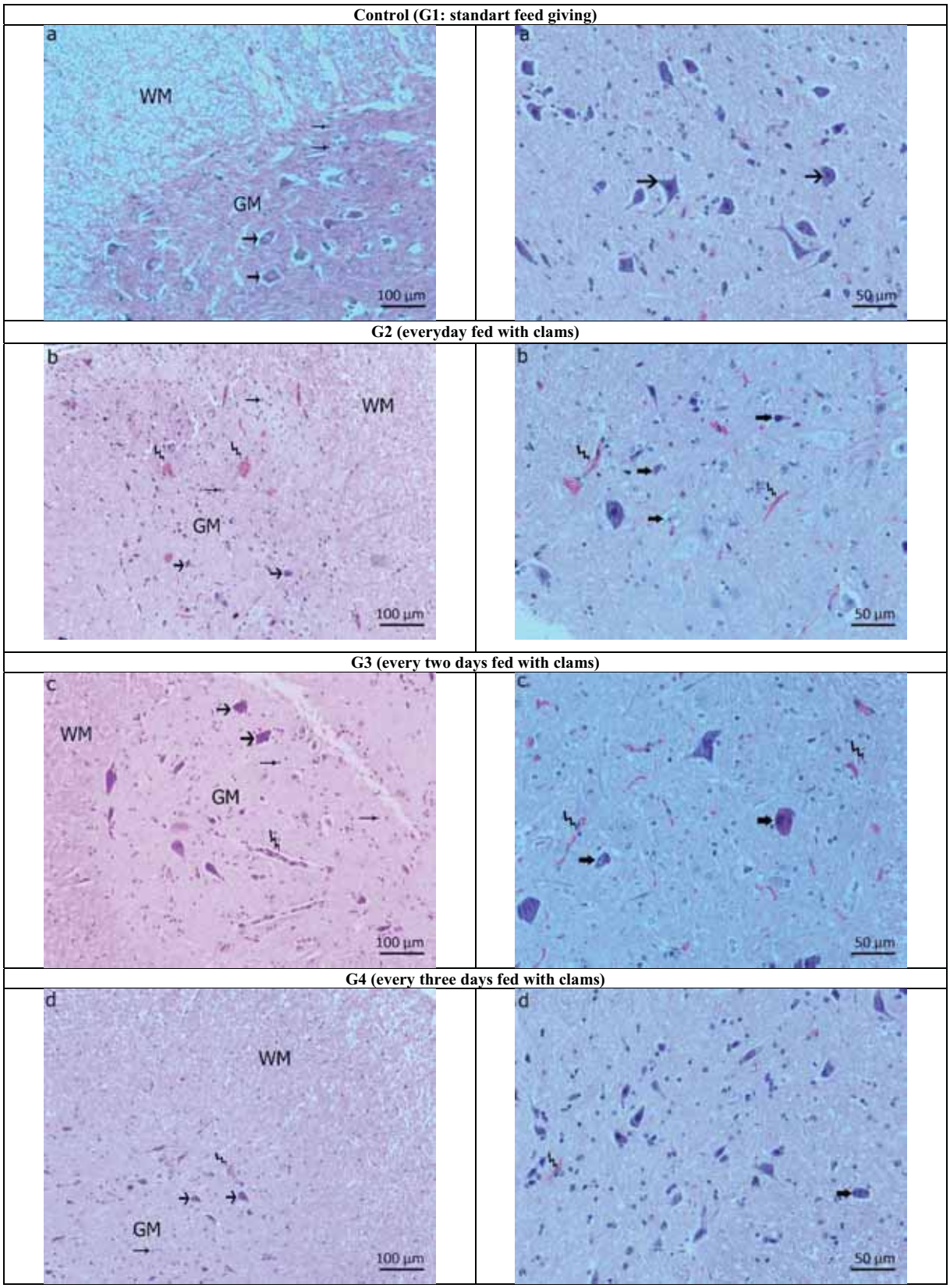


FIGURE 1

a) Control group spinal cord (WM: White mater, GM: Gray mater, arrow: multipolar neurons), b) Spinal cord of the second group giving clams everyday giving clams (long arrow: glial cells, short arrow: atrophic motor neurons degeneration, thunder: congestion), c) Spinal cord of the third group giving every two days clams (long arrow: glial cells, short arrow: arrow: atrophic motor neurons degeneration, thunder: congestion), d) Spinal cord of the fourth group giving every three days clams (Long arrow: glial cells, short arrow: atrophic motor neurons degeneration)

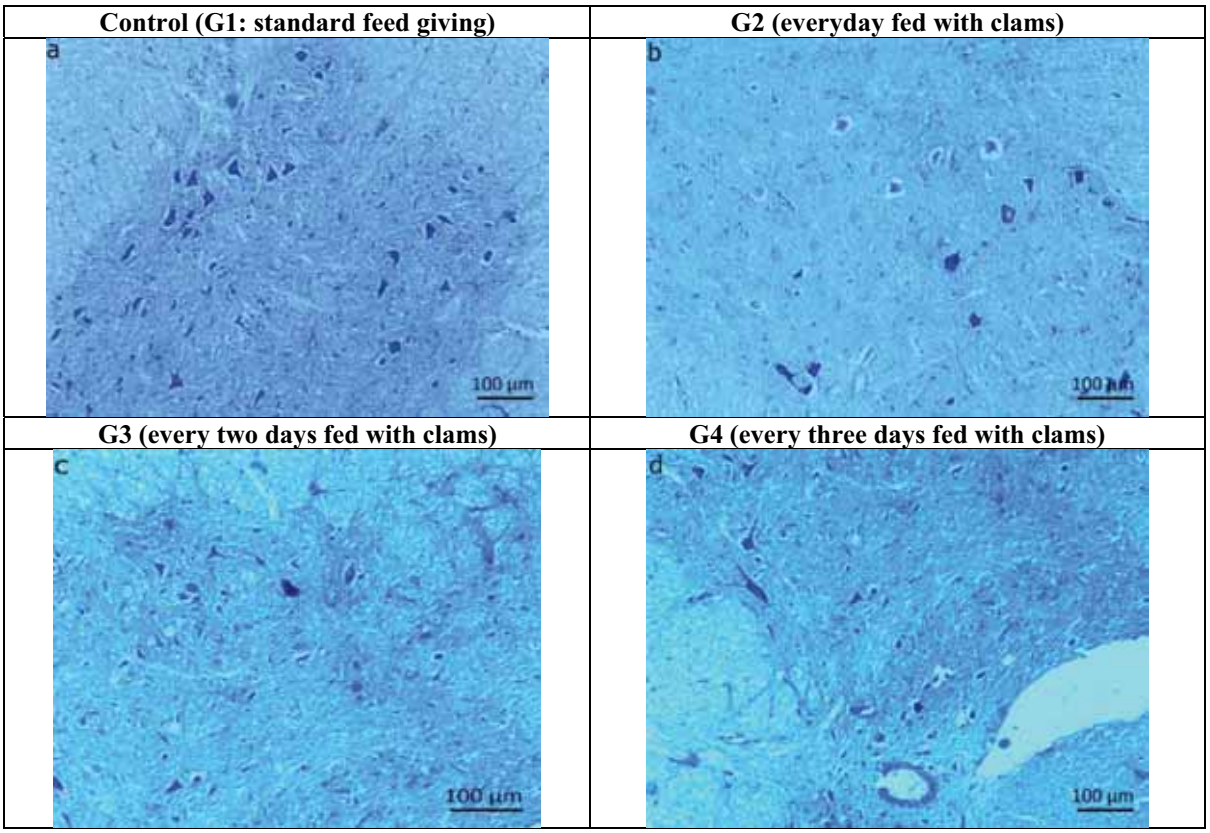


FIGURE 2
Toluidine blue staining of spinal cord of control (a) and other experimental groups (b, c, d). In the control group, the myelin sheaths have a normal shape. The myelin sheaths have a disorderly arrangement of other groups especially everyday fed with clams.

In addition, preparations stained with toluidine blue showed chromatolysis and demyelination. It has been found in previous research that heavy metals cause neuropathy in the nervous tissue. In this study, we observed that the rats fed with clams, which we thought were contaminated with heavy metals and other contaminating factors, had degeneration in the motor and sensory neurons due to the damage of glial cells in the spinal cord tissues.

TUNEL Assay Findings. In H&E staining, we observed that there were necrotic structures and apoptosis in many motor and sensory neurons. Spinal nerve cell necrosis was characterized by the fact that the neuronal cytoplasm turned into a homogeneous acidophilic structure and loss of cell functions. We performed staining with TUNEL method to

determine apoptotic cells. In the control group, we did not find motor and sensory neurons leading to apoptosis. But we observed that apoptosis occurs in glia cells. In the spinal cord of rats fed with clams, we detected that apoptosis occurred very strongly in glia cells, while a weak positivity occurred in the nucleus with DNA fractures in the motor and sensory neurons. This cellular structure suggests that glial cell damage can trigger neuronal damage when prolonged. We obtained findings proving that neuronal damage may increase when clam's delivery time is increased (Figure 3, Table 2).

Heavy Metal Analysis Results. According to the research findings, considering the Cd, Pb, Cu and Zn data in *T. decussatus*, the places where the heavy metal concentration is the highest are Camburnu, Yenikordon and Cardak stations, respectively (Table 1).

DISCUSSION

This study shows that reason apoptotic histological alterations in the spinal cord of rats long-term feeding with clam, is gliosis and demyelination in the spinal cord. We found differences in neurons and neuroglial morphology between the control group and the feeding clam 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].

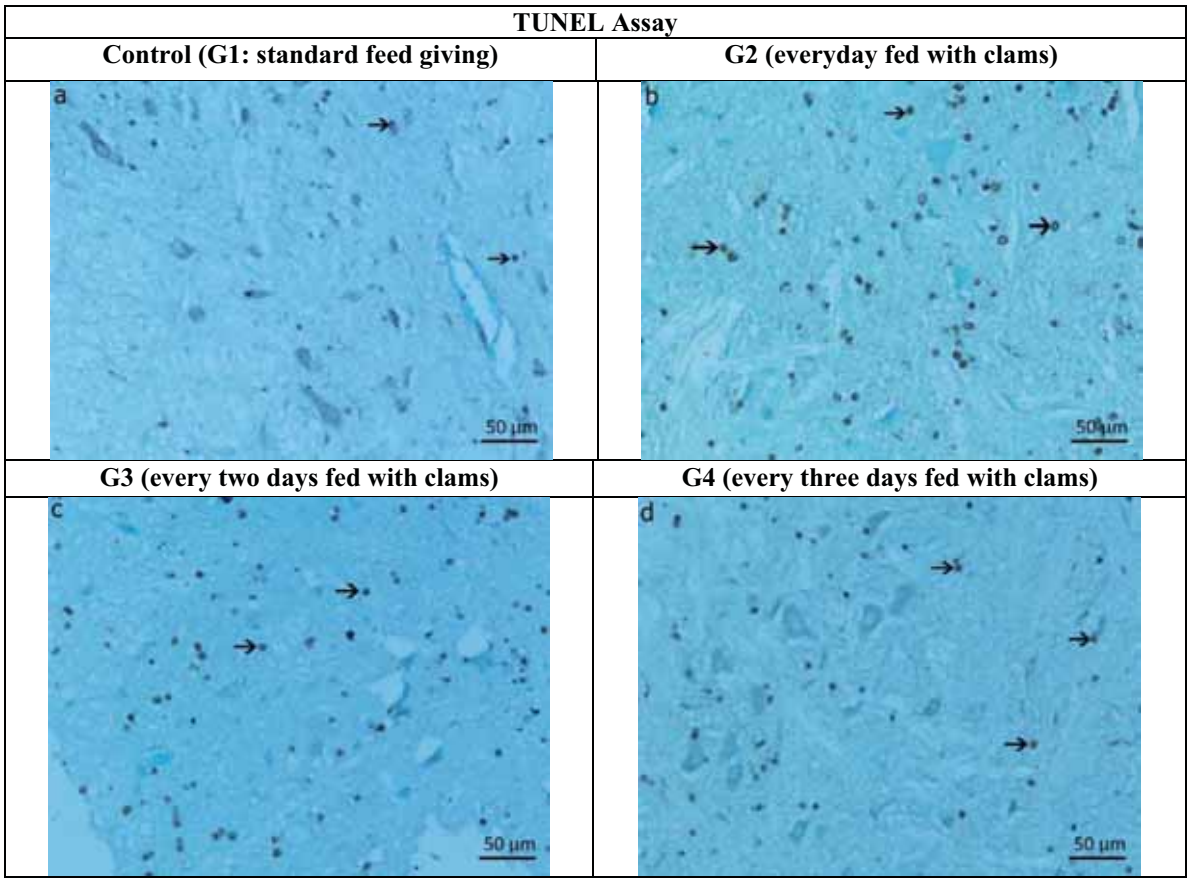


FIGURE 3
The TUNEL assay in the control (a) and experimental groups (b, c, d) (Arrow: apoptotic cells).

Apoptosis was more important in the glia cells for example oligodendrocyte of the clam 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 [18]. In this case, glial cells are the primarily affected cells in the neurodegenerative process induced by clams, showing a total chromatin fragmentation in white matter in contrast with gray matter.

In studies with transgenic mice, superoxide dismutase (SOD1) has been shown to reduce neurodegeneration in living organisms. This intoxication is thought to damage the motor neuron, but glia cells are thought to stimulate and support the motor neuron to prevent it [19, 20]. This support may help delay neuronal death with results showing that the apoptotic bodies are not as clear as the white matter in the gray matter because of the TUNEL technique in our study. In another study, however, histopathological changes of the spinal cord after intrathecal injection of tolfenamic acid included degeneration of nerve fibers, neuronal injuries in the form of degeneration, atrophy, necrosis, and apoptosis of the motor neuron [21]. In another study, the fish were exposed to herbicides and behavioral changes were observed. In the histopathology of the medulla spinalis, an increase in neuron loss, intracellular edema, vacuolization, deformation in Nissl granules and gliosis were detected [22]. It has been reported that neurodegeneration was severe in oligodendrocytes, myelin sheath weakened, and the number of neurons decreased but the number of astrocytes increased in the rat vertebral marrow fed by adding lead to drinking water for three months [23]. Our findings are obtained from the studies; It supports the findings of neurotoxicity that may occur because of irregular use of drugs and exposure of products such as fish and shellfish from sea and lakes to herbicides and heavy metals.

In this study, significant demyelination was observed in the treated group. This demyelination was confirmed by staining with Toluidine blue in rats fed with clams. Studies have reported that the damage to oligodendrocytes is a result of heavy metal exposure such as lead prevents myelin formation. In addition, because of apoptosis in oligodendrocytes, besides demyelination, gliosis and a rapid microglial response have been reported [24, 25]. Microglia cells are very active in the adult brain, and their activation is associated with morphological changes such as mobile branches and ameboid migration [26]. Moreover, microglial cells have a dual function and can be neurotoxic or neurotrophic depending on the specific stimulus, the severity of the injury, and the environment [27, 28]. When microglia were analyzed, no major morphological difference was observed between the control group and the clams-fed group.

Baskar Jesudasan et al. showed that the spinal cord microglia exhibited less inflammatory and phagocytic phenotypes than brain microglia in response to activation with lipopolysaccharides [28]. Typically, the identification of microglia in a normal environment would show branched morphology that transforms into an ameboid and spherical form when the medium deteriorates [29]. Activated microglia is neuroprotective against early amyloid deposition in Alzheimer's disease pathology [30. It has been found that microglial activity in the spinal cord is higher than the brain and possibly affects development in spinal cord diseases [31].

All heavy metals are potentially harmful at the level of exposure. Aquatic animals are also exposed to high levels of heavy metals. Heavy metals such as Cd, Pb, Hg, Zn are mainly found in mollusks and hepato-pancreas, gonads, and gills of shellfish. Therefore, clams have been used as biological indicator organisms to monitor marine pollution, especially as they accumulate heavy metals and potentially toxic chemicals in their bodies [32, 33]. It became important because most heavy metals are toxic at certain concentrations and can increase their concentrations during the transition from one organism to another. Clams can also filter toxic substances during water filtration. It floats through the food chains and can harm all living things, including humans [34].

In recent years, Turkey's coast has been studied for heavy metal accumulation in fish and other aquatic organisms. Marmara Sea, Aegean Sea, Black Sea and Mediterranean have been exposed to more heavy metal pollution due to industrial pollution from different facilities [35, 36] identified heavy metals in sea water and a large number of mollusks that grow in the Dardanelles. Based on this crustacean determination made earlier, regions with high amount of heavy metal were determined and Camburnu location was preferred. It was observed that the heavy metals measured in the muscle tissue of the clams we used as bait in our study were close to the results of the previous study. Heavy metals accumulate in living organisms and trigger stress factors, thus causing chronic damage in tissues with limited regenerative ability.

CONCLUSION

In conclusion, it has been found in previous research that heavy metals and pesticides cause neuropathy in the nervous tissue of living things. In this study, in addition to the findings of other researchers, long term clam feeding obtained from dirty environment induces neurodegeneration, apoptosis of oligodendrocytes, demyelination, and astrogliosis in the rat spinal cord. Our results suggest that demyelination and the astrogliosis process induce neuronal death in the spinal cord of rats feeding clam. The findings of other researchers and the results of our study regarding the toxic effects of heavy metals in living things reveal that the sea and lake areas where the seafood consumed grows should be free of all kinds of environmental pollution.

ACKNOWLEDGEMENTS

Contributing to the study during the evaluation of histopathological results, I would like to thank Dr. Samil Ozturk and Ilhan Ozdemir.

Declaration of conflicting interests. The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

REFERENCES

[1] Vanerkar, A.P., Satyanarayan, S., Dharmadhikari, D.M. (2004) Toxicity of herbal pharmaceutical wastewater on fish *Lebistes reticulatus* (Peter). J. Environ. Sci. Health B. 39, 115-123.

[2] Gezen, M. (2018) Histopathological changes in the stomach tissue of rats fed with mussels (*Mytilus galloprovincialis*). Journal of Scientific Perspectives. 2, 2-10.

[3] Varlık, B. (1991) Investigation of effects of some Heavy metal (Cd-Pb) to the different development stages of *Mytilus galloprovincialis* (Master's thesis) Dokuz Eylül University, İzmir.

[4] Kayhan, F., Muşlu, M., Koc, N. (2009) Stress and biological responses of some heavy metals on aquatic organisms. Journal of Fisheries Science. 3, 153-62. (in Turkish)

[5] Aslam, B., Javed, I., Khan, H. and Rahman, Z. (2011) Uptake of heavy metal residues from sewage sludge in the goat and cattle during summer season. Pakistan Veterinary Journal. 31, 75-7.

[6] Duffus, J. (2002) Heavy metals: a meaningless term [IUPAC Technical report]. Pure Appl. Chem. 74, 793-807.

[7] Park, J. and Zheng, W. (2012) Human exposure and health effects of inorganic and elemental mercury. J. Prev. Med Public Health. 45, 344-52.

[8] Özbolat, G. and Tuli, A. (2016) Effects of heavy metal toxicity on human health. Archives Medical Review Journal. 25(4), 502-521. (in Turkish)

[9] Fang, F., Peters, T., Beard, J., Umbach, D., Keller, J. and Mariosa, D. (2017) Blood lead, bone turnover, and survival in amyotrophic lateral sclerosis. Am. J. Epidemiology. 186(9), 1057-64.

[10] Baruah, J., Rasool, C.W., Bradley, W. and Munsat, T. (1981) Retrograde axonal transport of lead in rat sciatic nerve. Neurology. 31(5), 612-616.
[11] Pamphlett, R. and Ng, Y. (1998) Does lead damage motor neurons? Journal of Clinical Neuroscience. 5(1), 66-69.
[12] Roy, N., DeWolf, S. and Carneiro, B. (2015) Evaluation of the developmental toxicity of lead in the *Danio rerio* body. Aquatic Toxicology. 158, 138-48.
[13] Gomes, C., Cunha, C., Nascimento, F., Ribeiro, J., Vaz, A. and Brites, D. (2018) Cortical neurotoxic astrocytes with early ALS pathology and miR-146a deficit replicate gliosis markers of symptomatic SOD1G93A mouse model. Molecular Neurobiology. 56(3), 2137-2158.
[14] Siemer, D.D., Koteel, P. and Jariwala, V. (1976) Optimization of arsine generation in atomic absorption arsenic determinations. Analytical Chemistry. 48(6), 836-840.
[15] Gurpınar, T., Ekerbicer, N., Uysal, N., Barut, T., Tarakcı, T. and Tuglu, M.I. (2012) The effects of the melatonin treatment on the oxidative stress and apoptosis in diabetic eye and brain. Scientific World Journal. 2012, 498-489.
[16] Ozturk, S., Sonmez, P., Ozdemir, I., Topdagı, Y.E. and Tuglu M.I. (2019) Antiapoptotic and proliferative effect of bone marrow-derived mesenchymal stem cells on experimental asherman model. Cukurova Medical Journal. 44, 434-446.
[17] Dabrowska-Bouta, B., Struzynska, L., Walski, M. and Rafalowska, U. (2008) Myelin glycoproteins targeted by lead in the rodent model of prolonged exposure. Food and Chemical Toxicology. 46(3), 961-966.
[18] Ilieva, E., Ayala, V., Jove, M., Dalfo, E., Cacabelos, D. and Povedano, M. (2007) Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain. 130(12), 3111-3123.
[19] Boillee, S., Yamanaka, K., Lobsiger, C.S., Copeland, N.G., Jenkins, N.A., Kassiotis, G., Kollias, G. and Cleveland, D. (2006) Onset and progression in inherited ALS determined by motor neurons and microglia. Science. 312(5778), 389-392.
[20] Barbeito, A.G., Martínez-Palma, L., Vargas, M.R., Pehar, M., Many, N., Beckman, J.S., Barbeito, L. and Cassina P. (2010) Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS. Neurobiology of Disease. 37(3), 574-280.
[21] Abdelhakiem, M.A.H., Abdelbaset, A.E., Elkareem, M.A., Rawy, M.S. and Mahmoud, H.F.F. (2020) Clinical blood gas indices and histopathological effects of intrathecal injection of tolfenamic acid and lidocaine Hcl in donkeys. Comp. Clinical Pathology. 29, 83-93.
[22] Uyanikgil, Y., Yalcinkaya, M., Ates, U., Baka, M. and Karakis, H. (2009) Effects of 2,4-dichlorophenoxyacetic acid formulation on medulla spinalis of *Poecilia reticulata*: a histopathological study. Chemosphere. 76(10), 1386-1391.
[23] Villa-Cedillo S, Nava-Hernández M, Soto-Domínguez A, Hernández-Ibarra J, Perez-Trujillo J, Saucedo-Cárdenas O. (2019) Neurodegeneration, demyelination, and astrogliosis in rat spinal cord by chronic lead treatment. Cell Biology International. 43(6), 706-714.
[24] Ma, T., Wu, X., Cai, Q., Wang, Y., Xiao, L., Tian, Y. and Li, H. (2015) Lead poisoning disturbs oligodendrocytes differentiation involved in decreased expression of NCX3 inducing intracellular calcium overload. International Journal of Molecular Science. 16(8), 19096-19110.
[25] Caprariello, A.V., Mangla, S., Miller, R.H. and Selkirk, S.M. (2012) Apoptosis of oligodendrocytes in the central nervous system results in rapid focal demyelination. Ann. Neurology. 72(3), 395-405.
[26] Nimmerjahn, A., Kirchhoff, F. and Helmchen, F. (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 308(5726), 1314-1318.
[27] Lai, A.Y. and Todd, K.G. (2008) Differential regulation of trophic and proinflammatory microglial effectors is dependent on severity of neuronal injury. Glia. 56(3), 259-270.
[28] Jesudasan, B.S.J., Todd, K.G. and Winship, I.R. (2014) Reduced inflammatory phenotype in microglia derived from neonatal rat spinal cord versus brain. PLoS One. 9(9). Article ID: e99443.
[29] Zahn, J., Moller, T., Kettenmann, H. and Nolte, C. (1997) Microglial phagocytosis is modulated by pro- and anti-inflammatory cytokines. Neuroreport. 8(18), 3851-3856.
[30] vonderEmbse A.N., Hu, Q. and DeWitt, J.C. (2017) Developmental toxicant exposure in a mouse model of Alzheimer's disease induces differential sex-associated microglial activation and increased susceptibility to amyloid accumulation. Journal of Developmental Origins of Health and Disease. 8(4), 493-501.
[31] Olson, J.K. (2010) Immune response by microglia in the spinal cord. Annals of the New York Academy of Sciences, 1198, 271-278.
[32] Pempowiak, J., Sikora, A. and Biernacka, E. (1999) Specification of heavy metals in marine sediments, their bioaccumulation by mussels. Chemosphere. 39, 313-321.
[33] Hu, H. (2000) Exposure to metals. Prim. Care. 27, 983-996.

[34] Bat, L., Gundogdu, A., Ozturk, M. and Ozturk M. (1999) Copper, zinc, lead, and cadmium concentrations in the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck 1819) from the Sinop coast of the Black Sea. Turkish Journal of Zoology. 23, 321-326.

[35] Egemen, O., Alparslan, M. and Sunlu, U. (2004) Investigation of some heavy metal levels in mussels (*Mytilus galloprovincialis*) collected in Canakkale. Ege University Journal of Fisheries, 14, 189-196.

[36] Gezen, M.R., Demir, N. and Cetin, M. (2011) Investigation of heavy metal levels in sea water and some molluscs (Bivalvia and Gastropoda) on the Umurbey coast of the Canakkale Strait. Ecology Symposium. 2-11.

Received: **23.09.2020**
Accepted: **06.11.2020**

CORRESPONDING AUTHOR

Latife Ceyda Irkin
Canakkale Onsekiz Mart University,
Canakkale School of Applied Science,
Department of Fisheries Technology,
Canakkale – Turkey

e-mail: latifeirkin@gmail.com