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CONTENTS

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Immunohistochemical Investigation of Trk-A Receptor Levels in Pancreatic Tissue of Cumin (*Cuminum cyminum*) Plant Essential Oil Treated-Mice

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Abstract

This study was conducted to immunohistochemically investigate Trk-A receptor levels in pancreas tissue of mice treated by cumin (*Cuminum cyminum*) plant essential oil. Mice were grouped into control group (n = 10) and trial group (n = 10). No application was performed to the mice in the control group. The mice in trial group were treated by 500 mg/kg of oral cumin essential oil every 24 hours for two days. At the end of study, the pancreatic tissues obtained were blocked in paraffin following routine histological processes. Triple staining was performed to the sections taken from these blocks to examine general histological structure of pancreas. Acinus, islets of Langerhans, pars initialis, pars excretory and ductus excretorius were determined in mice pancreas. Immunohistochemical studies showed that all mice had Trk-A immunoreactivity in pancreatic tissue. Moderate immunoreactivity in acini and weak immunoreactivity in islets of Langerhans and excretory ducts were detected in pancreas tissue of mice in control and trial groups. It was determined that there was no difference between the groups in terms of Trk-A immunoreactivity in acini and islets of Langerhans. Based on the immunohistochemical results, cumin was used in field of diuretic, degassing, digestion facilitator, antimicrobial and antidiabetic effects in field of traditional medicine; It was concluded that Trk-A receptor synthesized from pancreatic tissue does not change its levels.

Keywords: Cumin, pancreas, Trk-A.

1. INTRODUCTION

Plants have been used for various purposes such as treatment of diseases, defense and nutrition since the past. Today, people continue to benefit from these effects of plants but the use of plants is more consciously done. Plants are used in many fields such as food, pharmacy, agriculture and medicine (Göktaş and Gıdık, 2019). Cumin (*Cuminum cyminum*) is a plant also known as Persian cumin, Avcar or Kemmon. Its native soil is Egypt, but is raised in Mediterranean countries and Middle Anatolian Region of Turkey as well. In addition to its

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usage as a spice, it has been used as carminative and diuretic, reliever of stomach discomfort and exudative (Baytop, 1999). Cumin was reported to have beneficial effects on patients with obesity and metabolic syndrome (Fernando, Perera, Athauda, Sivakanesan, Kumar, Jayasinghe, 2019; Morovati, Pourghassem, Sarbakhsh, Azari and Lotfi-Dizaji, 2019).

Cumin has been reported to may have positive effects on cancer treatment disadvantages and antimicrobial resistance (Nirmala, Durai, Rao and Nagarajan, 2020). Trk-A receptor belongs to the family of thyrosine kinase which has a role in the development of peripheral nervous system (Kiriş, Wang, Yanpallewar, Dorsey, Becker, ….Tessarollo, 2014). It has been stated that Trk-A may have effects on insülin and glucose metabolism with its synthesis in the pancreas, other than the nervous system (Rosenbaum, Sánchez-Soto and Hiriart, 2001). NTRK gene fusions involving either NTRK1, NTRK2 or NTRK3 (encoding the neurotrophin receptors TRKA, TRKB and TRKC, respectively) have important roles in diagnostic evaluation of central nervous system tumors and pediatric mesenchymal tumors (Gambella, Senetta, Collemi, Vallero, Monticelli, …. Bertero, 2018; Rudzinski, Lockwood, Stohr, Vargas, Sheridan, ….Davis, 2020). In addition, It is also considered as a biomarker in digestive system cancers, including esophageal, stomach and pancreatic cancers (Blondy, Christou, David, Verdier, Jauberteau, Mathonnet and Perraud, 2019). It was reported that Trk-A immunolocalization in the pancreas different in islet cells and ductal cells throughout fetal life. Trk-A immunoreactivity was observed to be weaker in islet cells than in adult rats in the early postnatal period. In addition, that as the maturation of the pancreas progresses, the Trk-A immunoreactivity was reported in the ducts gradually decreases. Therefore, it was stated that the intensity and localization of Trk-A immunoreactivity in pancreatic tissue are regulated developmentally (Kanaka-Gantenbein, Tazi, Czernichow and Scharfmann, 1995). In pancreatic tissue, Trk-A immunoreactivity has been reported to be strong granular in the cytoplasm of acinar cells and weak cytoplasmic in langerhans islets (Yediel-Aras, 2016). This study was carried out to immunohistochemically investigate Trk-A receptor levels in the pancreas tissue of cumin (*Cuminum cyminum*) plant essential oil treated-mice.

2. MATERIALS AND METHOD

Approval for the research was received from Kafkas University Animal Experimentation Local Ethics Committee (KAÜ-HAYDEK No: 2017-071). Laboratory animals used in the research were supplied from Atatürk University Laboratory Animals unit.

2.1. Animals

Eight-week-old, twenty female mice (*Mus musculus*) weighing 20 ± 1 gr. were used in the study. Mice were grouped into control group (n = 10) and trial group (n = 10). Mice were fed ad libitum and were given free access to tap water. No intervention was performed to the mice in the control group. Oral gavage of cumin volatile oil per 24 hours for two days was applied to the trial group mice depending on their weight as 500 mg/kg (Rodrigues-Alves, Souza dos Santos, Calil, Niero, Lopes, …. Maistro, 2014). At the end of the study, mice were sacrificed under deep anesthesia, and obtained pancreas tissue samples.

2.2. Preparation of cumin extract

Cumin plant was with distilled water vapour with Non-Asbestos brand Clevenger device for 3,5- 4 hours by adding 40 grams of plant and 400 ml of water. At the end of 4 hours volatile oil was stored in drip pan and at +4˚C.

2.3. Histological examinations

Pancreas tissue samples were fixed within 10% formalin solution. Following routine procedures, they were embedded into paraffin blocks, and 5 μm sections were obtained. In order to demonstrate histological structure of pancreatic tissue, the sections were performed Crossman's Triple Staining (Luna, 1968) staining methods.

2.4. Immunohistochemical investigations

The Avidine-Biotin-Peroxidase technique was applied to the tissue samples to investigate Trk-A immunoreactivity (Hsu, Raine and Fanger, 1981). The slides were incubated in 3% H_20_2 (hydrogen peroxide) prepared in 0.1 M phosphate buffered saline (PBS) for 15 min, in order to inhibit endogenous peroxidase activity after deparaffinization and rehydration procedures. Then slides were washed in PBS solution and for releasing the antigenic receptors was boiled in microwave at 600 watts for 10 minutes in a tris- EDTA Buffer solution (pH: 6.0). Tissues were incubated with Blocking Solution A (Invitrogen-Histostatin Plus Bulk Kit) for 10 minutes and then Trk-A (Abcam-AB76291) (1/400 dilution) primary antibody at room

temperature for an hour. Biotinylated Secondary Antibody and Streptavidin-Peroxidase solutions were applied to the slides 30 minutes. DAB-H_2O_2 (Diamino benzidine hidrogen peroxide) (Shu, Ju and Fan, 1988) was applied to the PBS washed tissues as an encolouring substrate. After adding a chromogen solution on tissues, the reaction was terminated with PBS depending on the status of immunoreactivity by controlling under the light microscope. Hematoxylin was applied on tissues for counterstaining after washing with distilled water. Tissues were then dehydrated and covered with immunmount. Staining level was accepted as a criterion and scoring were performed by semi quantitative method. The evaluation was made by two independent observers. Depending on the staining properties, tissues were scored within the range of 0-3 during their evaluation: weak staining (1), intermediate staining (2), intense staining (3) (Zhu, 1989; Seidal, Balaton and Battifora, 2001). In order to test the specificity of immunohistochemical staining, the same procedures without adding antibodies (negative control) were applied to pancreatic tissue of mice in all groups. Tissues prepared for histological and immunohistochemical investigations were evaluated and photographed with a light microscope (Olympus BX51; Olympus Optical Co. Osaka, Japan). The number of Trk-A immunopositive cells were count using the image-j (vI. 50i) software. Numerical distribution of Trk-A positive cells were observed in ten different sections chosen from ten unit area of Langerhans islet and acinar cells of each animals (Eliş-Yıldız, Yediel-Aras, Dağ ve Karadağ-Sarı, 2019).

2.5. Statistical analysis

SPSS (20.0) package software was used to evaluate the data obtained in the study. T test was used to determine the difference between the groups on account of Trk-A immunoreactivity.

3. RESULTS

It was observed that the acinus, islets of Langerhans, pars initialis, pars excretory and ductus excretorius of the pancreatic tissues of control and trial groups in normal histological structure (Figure 1 A-B). Trk-A expression was observed both in the endocrine cells (islets of Langerhans) and in the exocrine cells in mice pancreas of two groups. Trk-A immunoreactivity was observed in acinus, islets of Langerhans and ductus excretorius in mice pancreatic tissues of control and trial groups. Intermediate immunoreactivity was detected in the cytoplasm of acinar cells in pancreatic tissues of control group mice. Weak diffuse cytoplasmic Trk-A immunoreactivity was determined in islets of Langerhans and ductus excretorius (Figure 2-A).

Regions that show Trk-A immunoreactivity findings in pancreatic tissues of the mice revealed similarities with regards to control and trial groups. Intermediate diffuse cytoplasmic Trk-A immunoreactivity was detected in acinar cells whereas islets of Langerhans and ductus excretorius showed weak diffuse immunoreactivity (Figure 2-B).

Count of Trk-A immunoreactivity positive cells acini and Langerhans island in among groups were summarized in Table 1-2. There was no difference between the groups in terms of Trk-A immunoreactivity in acinus and Langerhans islets (p>0.05).

Table 1. Count of Trk-A immunoreactivity positive cells acini in among groups.

Group	N	Mean	Std. Deviation	P
Control	10	400.5	18.02	0.979
Trial	10	400.7	15.98	

There was no difference between the groups in terms of Trk-A immunoreactivity in acinus (p>0.05).

Table 2. Count of Trk-A immunoreactivity positive cells Langerhans island in among groups.

Group	N	Mean	Std. Deviation	P
Control	10	88.6	16.243	0.974
Trial	10	88.4	9.845	

There was no difference between the groups in terms of Trk-A immunoreactivity in Langerhans islets (p>0.05).

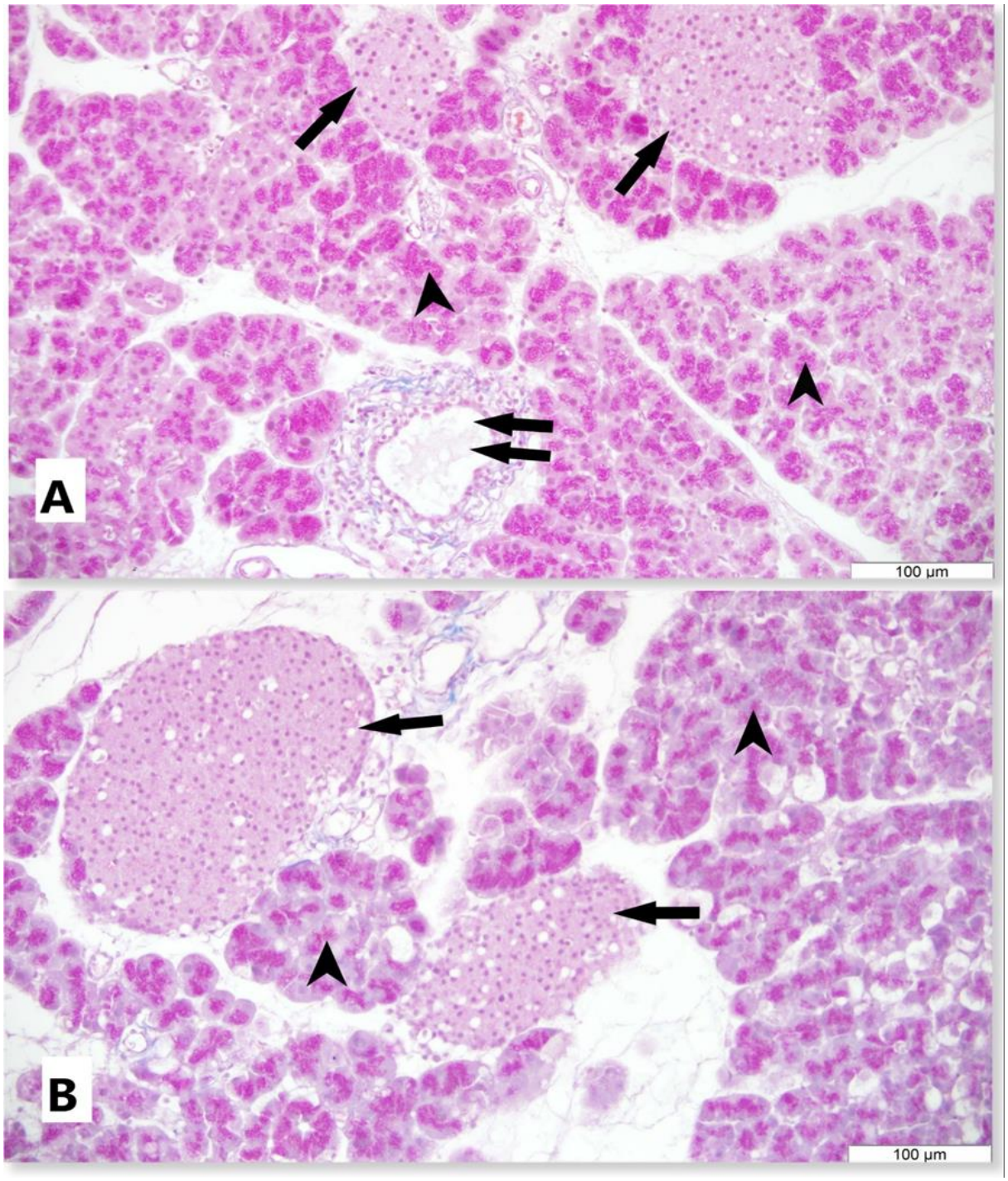

Figure 1. Mice pancreatic tissue. A: Control group, B: Trial group. Arrow: Langerhans island, arrowhead: acini, double arrow: pars excretoria. Triple staining.

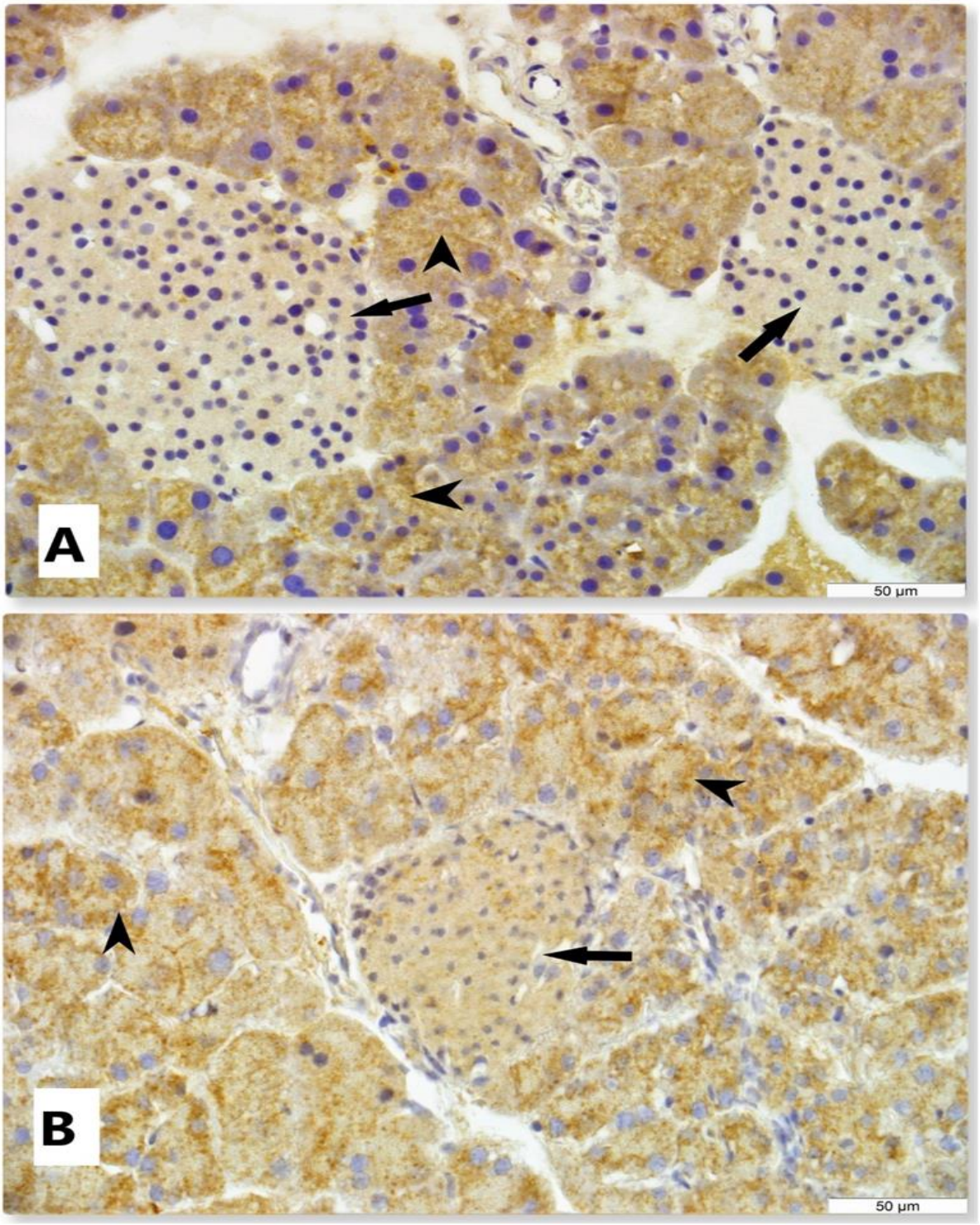

Figure 2. Mice pancreatic tissue Trk-A immunoreactivity. A: Control group, B: Trial group. Arrow: Langerhans island, arrowhead: acini.

4. DISCUSSION AND CONCLUSION

Based on the investigation of cumin on weight loss of overweighed people, high dose cumin application for 8 weeks has positive effects on hight weight, body mass index, total cholesterol and LDL levels (Taghizadeh, Memarzadeh, Abedi, Sharifi, Karamali, Keşan and Asemi, 2016). Cumin was reported to have a preventive effect as a natural antioxidant against experimental hepatotoxicity in mice (Sheweita, El-Hosseiny and Nashashibi, 2016). In a research investigating the antioxidant effects of plants, it was stated that cumin might be used as a natural antioxidant resource in food and it might be effective in diminishing the complications of diabetes (El-Ghorab, Nauman, Anjum, Hussain and Nadeem, 2010; Keihan, Gharib, Momeni, Hemati and Sedighin, 2016). Cumin was also reported to have roles on improvement of immune system in diabetes patients, to diminish IgE to a level close to normal and to decrease cytokine and total blood count levels (Moubarz, Embaby, Doleib and Taha, 2016) Cumin was reported to have hypolipidemic effect on obese rats (Haque and Ansari, 2018).

It was stated that the cumin has antimicrobial and antioxidant activities (Haşimi, Tolan, Kızıl and Kılınç, 2014) and at a result of the investigation on the effect of cumin on pancreatic digestion enzymes, it was notified that cumin used with diet have stimulative effect on amylase, trypsin and chymotrypsin and it might have stimulating effect on digestion (Platel and Srinivasan, 2000). Trk-A immunoreactivity was detected in insulin secreting cells of Langerhans islets and pancreatic duct, and decreasing of Trk-A levels in pancreatic tissue after the occurrence of diabetes was reported (Miralles, Philippe, Czernichow and Scharfmann, 1998; Sposato, Manni, Chaldakov and Aloe, 2007). However Shibayama and Koizumi (1996) did not encounter Trk-A immunoreactivity in acinar cells and Langerhans islets and reported weak immunoreactivity in pancreatic duct. In our research, we detected Trk-A immunoreactivity in acinar cells, Langerhans islets and excretory duct of pancreatic tissue in mice pancreas on both control and trial groups. Trk-A immunoreactivity levels of pancreatic tissue of mice in control and trial groups was the same. In our study, the determination of Trk-A immunoreactivity in Langerhans islets was parallel with Miralles et al., 1998 and Sposato et al., 2007 data. However, Trk-A immunoreactivity was also detected in asinus of pancreas in our study. These results set us think that Trk-A may have roles on the development of pancreatic tissue and synthesis and secretion of various pancreatic hormones. It was also thought that the application dose and duration of cumin may cause similar Trk-A immunoreactivity in the control and trial groups.

In conclusion, cumin is a plant that benefits from diuretic, degassing, digestive facilitating, antimicrobial and antidiyabetic effects in the field of traditional medicine. In our

study, Trk-A receptor secretion was close to each other in the control and trial groups that has important roles in the regulation on the neural development and insulin metabolism of the pancreas. We think that gathered data will contribute to other researches that investigate the effects of cumin on pancreatic tissue related disease.

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Hirshfeld Surface Analysis and Interaction Energy Calculations of Bis(4-chlorophenylacetate)bis(pyridine-4-carboxamide)Zinc(II)

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Abstract

Hirshfeld surface analysis has been widely used in crystallography in recent years to investigate intermolecular interactions and to determine the contribution of these interactions to the crystal lattice. Fingerprint plots, which are given as color plots, present quantitative results of the types of intermolecular interactions in a molecule. In this study, we have been investigated intermolecular interactions and energy frameworks pyridine 4-carboxamide complex of Zn(II) 4-chlorophenylacetate by using CrystalExplorer program. Intermolecular interactions of the complex were determined using Hirshfeld Surface analysis. The intermolecular interaction energies of the complex were calculated using CE-HF/3-21G, CE-HF/6-31G (d), CE-HF/6-31G (d,p), CE-MP2/3-21G, CE-MP2/6-31G (d), CE-MP2/6-31G (d,p), CE-B3LYP/3-21G, CE-B3LYP/6-31G (d), CE-B3LYP/6-31G (d,p), CE-DFT/3-21G, CE-DFT/6-31G (d) and CE-DFT/6-31G (d,p) energy models that involved in CrystalExplorer (CE) program. The estimation of the intermolecular interactions and energies of the complexes is very important for the classification and investigation of their application areas.

Keywords: 4-Chlorophenylacetatic acid, pyridine-4-carboxamide, Hirshfeld Surface analysis, Energy Calculations, CrystalExplorer program.

1. INTRODUCTION

In recent years, theoretical chemistry has get importance in researching the structural, physical and biological properties of materials. The results obtained from theoretical studies help to estimate and interpret the results that can be obtained from experimental results, and it is much cheaper and faster in obtaining theoretical results compared to experimental results. For these reasons, to avoid from squander of money and time for experimets, the making of the theoretical calculations before the experiments is very important (Kirste, 2016; Toy, Tanak ve Şenöz, 2020). CrystalExplorer program is the most used theoretical calculation program, that it has been widely used by Crystallographs, in recent years (Turner, McKinnon, Wolff, Grimwood, Spackman, Jayatilaka, Spackman, 2017). Hirshfeld Surface analysis is an analysis that can leads to determination of the interactions among molecules, and the contribution of

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these interactions to the crystal lattice, by creating graphs and two-dimensional fingerprint plots. Thanks to this analysis, by determining the surface of Hirshfeld, it is possible to separate the electron density of the crystal into molecular parts and to describe the area in which the molecule is located. In this way, it can be detemined the similarity and/or differencies among structurally investigated crystal structures. With this analysis, it is possible to define not only the contribution of intermolecular interactions to crystal packing but also the characteristics of different intermolecular interactions (Tan, Jotani and Tiekink, 2019). That, is, Hirshfeld surface analysis helps to investigate the role of non-covalent interactions such as hydrogen bonds and other intermolecular contacts and to determine the effects of these interactions on crystal lattice stability. A new feature has been added to the Crystal Explorer 17.5 Program to allow calculation of pair-wise interaction energies within a crystal. Users can apply the energy models available in the software to perform the calculation for neutral organic molecules, organic salts, solvates, coordination compounds, and radicals (Hirshfeld, 1977; McKinnon, Jayatilaka and Spackman, 2007; Spackman and Jayatilaka, 2009). Within the scope of this study, the Hirhsfeld Surface analysis and Interaction Energy Analysis of the bis(4-chlorophenylacetate)bis(pyridine-4-carboxamide)zinc(II) complex, which we previously synthesized and determined its structure, were performed using the CrystalExplorer program.

In order to interpret the interactions among the molecules of the complex, d_{norm} map, shape index, curvedness map, 2D fingerprint plots and fragment patches of the molecule were determined. For the energy frameworks analysis of the complex, electrostatic energy, polarization energy, dispersion energy, exchange-repulsion energy and total intermolecular energy were computed using twelve energy models which were notified as: CE-HF/3-21G, CE-HF/6-31G (d), CE-HF/6-31G (d,p), CE-MP2/3-21G, CE-MP2/6-31G (d), CE-MP2/6-31G (d,p), CE-B3LYP/3-21G, CE-B3LYP/6-31G (d), CE-B3LYP/6-31G (d,p), CE-DFT/3-21G, CE-DFT/6-31G (d) and CE-DFT/6-31G (d,p).

2. MATERIALS AND METHOD

To investigated the visualization of intermolecular interactions of bis(4-chlorophenylacetate)bis(pyridine-4-carboxamide)zinc(II) complex, that we previously synthesized and characterized its structure (Figure 1) (Özbek, Sertçelik, Yüksek, Uğurlu, Tonbul, Necefoğlu and Hökelek, 2019) Hirshfeld surface analysis (Hirshfeld, 1977; Spackman and Jayatilaka, 2009) was carried out. Hirshfeld surface (Turner et.al., 2017; McKinnon et al., 2007), 2D fingerprint (Spackman and Jayatilaka, 2009) plots and intearction energy analysis were obtained using CrystalExplorer Version 17.5 program based on the input CIF (Turner et

al., 2017). To get more accuracy about molecular interactions, we used Tonto quantum chemistry package for energy framework analysis (Jayatilaka and Grimwood, 2003; Mackenzie, Spackman, Jayatilaka and Spackman, 2017; Tan et al., 2019). The intermolecular interaction energies of for bis(4-chlorophenylacetate)bis(pyridine-4-carboxamide)zinc(II) complex were computed using CE-HF/3-21G, CE-HF/6-31G (d), CE-HF/6-31G (d,p), CE-MP2/3-21G, CE-MP2/6-31G (d), CE-MP2/6-31G (d,p), CE-B3LYP/3-21G, CE-B3LYP/6-31G (d), CE-B3LYP/6-31G (d,p), CE-DFT/3-21G, CE-DFT/6-31G (d) and CE-DFT/6-31G (d,p) energy models in Crystal-Explorer (CE).

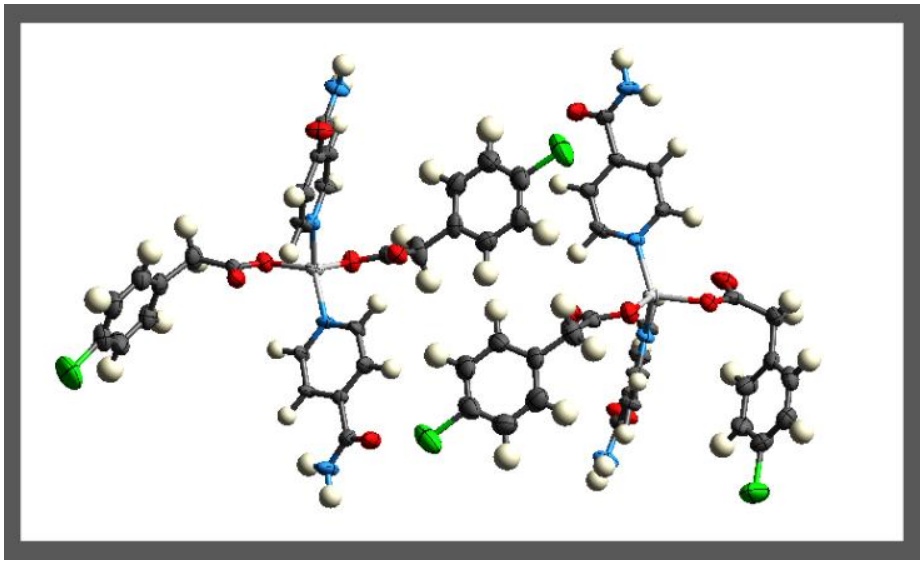

Figure 1. Crystal Structure of the complex

3. RESULTS AND DISCUSSION

3.1. Hirshfeld Surface Analysis

Hirshfeld surface analysis enables intermolecular interactions and short or long contacts to be visualized by presenting them with different colors and color intensity. The d_{norm} maps of the complex were given in Figure 2. In the d_{norm} map of the Hirshfeld Surface of complex 1, red and blue surfaces represent the contacts with distances shorter (in close contact) or longer (distinct contact) than the Van der Waals radii, respectively. In addition the white surface indicates the distance equal to the sum of Van der Waals radii. Three-dimensional Hirshfeld surfaces of the complex were plotted over d_{norm} in the range of -0.5875 to 1.6094 a.u. (Spackman, McKinnon and Jayatilaka, 2008).

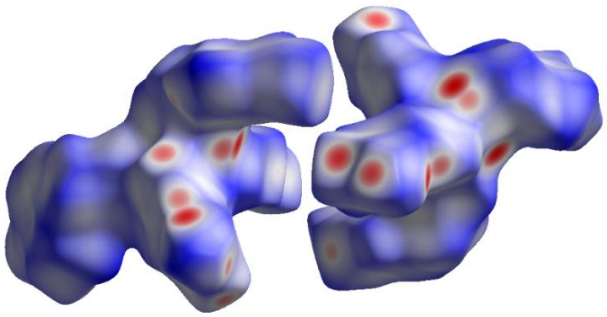

Figure 2. View of the three-dimensional Hirshfeld surface of the complex which were plotted over d_{norm} in the range -0.5875 to 1.6094 a.u.

On the shape-index for the complex, blue and red regions represent donor and acceptor groups, respectively (Figure 3). As can be seen from the Figure 3, adjacent red and blue triangles confirm the presence of π-π stacking interactions among the aromatic rings in the crystal structure of the complex (Spackman et al., 2008).

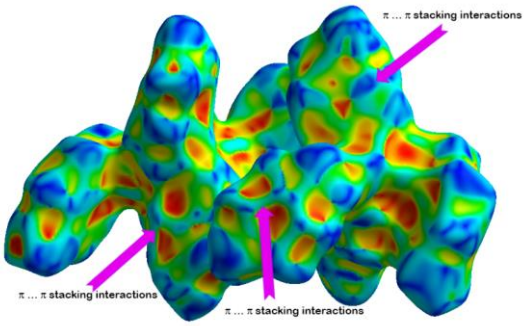

Figure 3. Shape-index plot in Hirshfeld surface of the complex.

There are showed relatively large green planes in where the benzene and pyridine rings ligands are placed. The curvedness mapping gives an idea of the planarity of complexes and that give rise to the π···π interactions between the benzene and pyridine rings in a monoclinic crystal system (Figure 4) (Spackman et al., 2008).

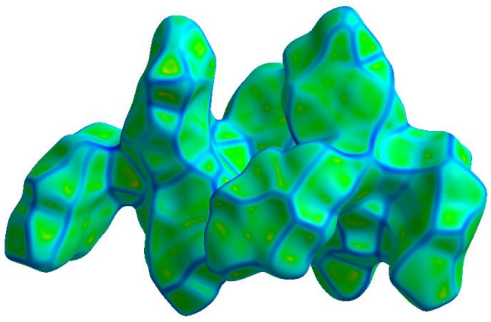

Figure 4. Curvedness mapping of the complex

2D fingerprint plots and fragment patches for all interactions and individual for each interaction are given in Figure 5. The most significant interactions are H···H (33%) interactions due to the abundance of hydrogen on the molecular surface. The second largest contribution (22.8%) was estimated as H···O/O···H interactions, which was consisted of the appearance of deep-red color and relationship with the N—H···O and C—H···O hydrogen bonds. In addition, H···C/C···H (16.9%), H···Cl/Cl···H (12.2%), C···Cl/Cl···C (4.3%), C···C (4.1%), H···N/N···H (2.5%), O···Cl/Cl···O (1.5%) and C…O/O…C (1.2%) interactions were also observed, with other contact types making a negligible contribution (Cl···Cl (0.8%) N···Cl/Cl···N (0.3%) and N···C/C···N (0.3%) (Figure 6).

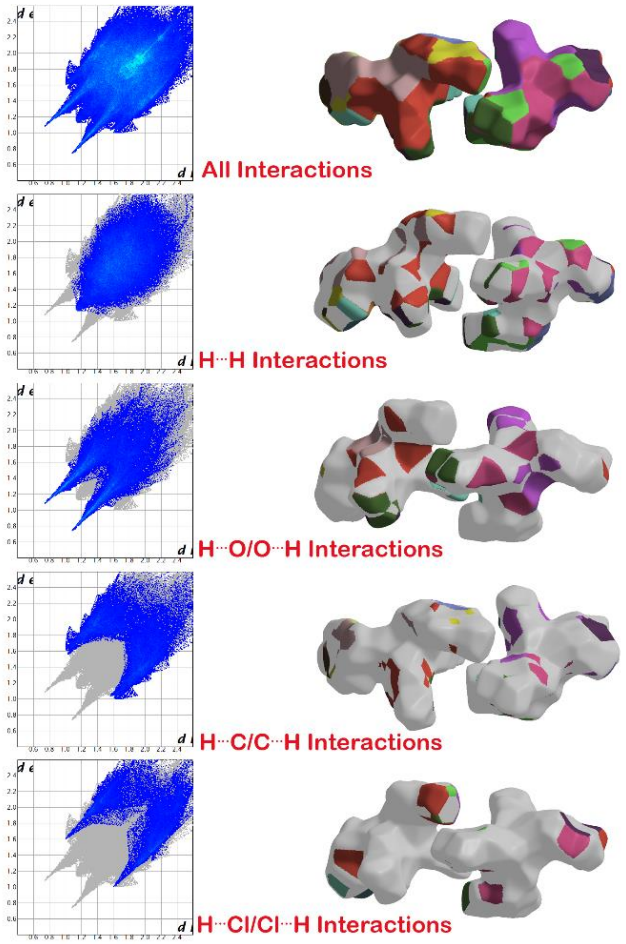

Figure 5. The Hirshfeld surface representations with the function dnorm which were plotted onto the surface.

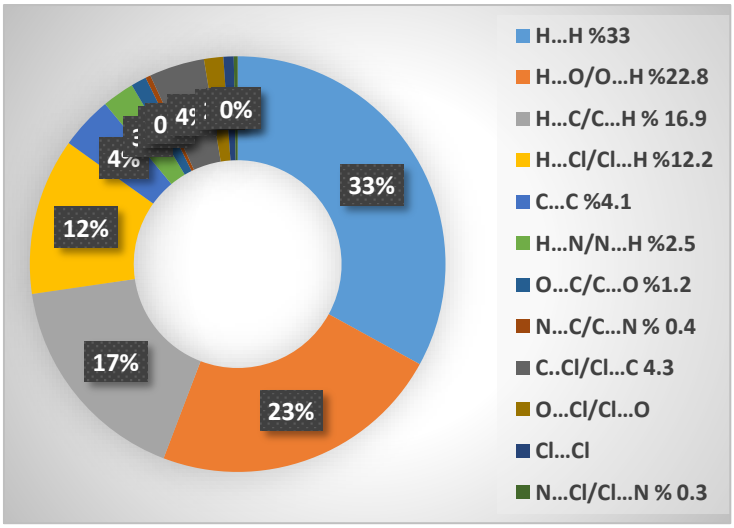

Figure 6. The percentages of the intermolecular interactions which were obtained from Hirshfeld Surface analysis of the complex.

3.2. Interactions Energy Analysis

The total intermolecular energy, E_{tot} (kJ/mol), which correlative to the reference molecule is the sum of four main energy components comprising electrostatic (E_{ele}), polarization (E_{pol}), dispersion (E_{dis}) and exchange-repulsion (E_{rep}) with scale factors (Tan et al. 2019) are given in Table 1. The energy calculation results which were taken in the Table 2, were done by using CE-HF/3-21G, CE-HF/6-31G (d), CE-HF/6-31G (d, p), CE-MP2/3-21G, CE-MP2/6-31G (d), CE-MP2/6-31G (d, p), CE-B3LYP/3-21G, CE-B3LYP/6-31G (d), CE-B3LYP/6-31G (d, p), CE-DFT/3-21G, CE-DFT/6-31G (d) and CE-DFT/6-31G (d, p) models of the program. Twelve different model of the CrystalExplorer (CE) program were used to increase the accuracy of the estimation of the energy values. Each energy model has showed very similar results in itself (Table 2). It was clearly seen from the Table that the dispersion (E_dis), polarization (E_pol) and exchange-repulsion (E_rep) energy values were obtained same for all of the models. This situation can be attributed to the stability of used reference molecule. On the other hand, the electrostatic energy (E_ele) values were generally found different and they affected the values of the total energies. In previous studies, it has been reported that hydrogen bond interactions contribute to electrostatic energy. Therefore, hydrogen bond interactions in crystal structure of the complex form as: N—H···O and C—H···O and the contributions to the electrostatic energies cause mainly from these interactions. The fact that the doesn't change of the dispersion energy is an indicator of the stability of the molecule Especially H…H interactions, which were found to have a large contribution among the layers by Hirshfeld surface analysis of the complex, are thought to significantly increase the stability

of the structure (Caracelli, Zukerman-Schpector, Schwab, Silva, Jotani and Tiekink, 2019; Etse, Lamela, Zaragoza and Pirotte, 2020; Mackenzie et al., 2017; Madan-Kumar, 2019).

Table 1. Scale factors for benchmarked energy models (Mackenzie et al., 2017)

Energy Model	k_ele	k_pol	k_disp	k_rep
CE-HF ... HF/3-21G electron densities	1.019	0.651	0.901	0.811
CE-B3LYP ... B3LYP/6-31G(d,p) electron densities	1.057	0.740	0.871	0.618

Table 2. Interactions energy analysis results

N	Symop	R	Electron Density	E_ele	E_pol	E_dis	E_rep	E_tot
1	-	12.03	HF/3-21G	3.4	-0.2	-3.2	0.0	0
1	-	12.03	HF/6-31G(d)	3.1	-0.2	-3.2	0.0	-0.3
0			HF/6-31G(d,p)	3.1	-0.2	-3.2	0.0	-0.3
1	-	12.03	MP2/3-21G	3.4	-0.2	-3.2	0.0	0
0			MP2/6-31G(d)	3.1	-0.2	-3.2	0.0	-0.3
0			MP2/6-31G(d,p)	3.1	-0.2	-3.2	0.0	-0.3
1	-	12.03	B3LYP/3-21G	2.5	-0.2	-3.2	0.0	-0.9
0			B3LYP/6-31G(d)	2.4	-0.2	-3.2	0.0	-1.0
1	-	12.03	B3LYP/6-31G(d,p)	2.4	-0.2	-3.2	0.0	-1.0
1	-	12.03	DFT/3-21G	2.3	-0.2	-3.2	0.0	-1.1
0			DFT/6-31G(d)	2.2	-0.2	-3.2	0.0	-1.2
1	-	12.03	DFT/6-31G(d,p)	2.2	-0.2	-3.2	0.0	-1.2

E:interaction energies components, Symop: rotational symmetry operations with respect to the reference molecule, R: the centroid-to-centroid distance between the reference molecule N: interacting molecules as well as the number of pair(s) of interacting molecules with respect to the reference molecule (Mackenzie et al., 2017).

4. CONCLUSION

In this study, Hirshfeld surfaces and the relationship 2D fingerprint plots of complex were investigated. According to Hirshfeld Surface Analysis results, H...H, H...C/C…H, H···Cl/Cl···H, C···Cl/Cl···C, C···C, H···N/N···H, O···Cl/Cl···O, C···O/O···C, Cl···Cl and N···Cl/Cl···N

interactions are found. H···H interactions provide the most contribute to crystal packaging. H···O/O···H interactions also provide the second most important contribution, support the presence of hydrogen bonds in the crystal structure. In addition to, this confirms the contribution of N—H···O and C—H···O hydrogen bonds to the stability of three-dimensional networks in the crystal package, previously obtained by single crystal X-ray diffraction. On the shape-index of the complex, presences of adjacent red and blue triangles were supported the π-π stacking interactions between the benzene and pyridine rings in complex's crystal structure. As a result, it can be said that the results obtained from single crystal X-ray analysis and Hishfeld surface analysis are supportive of each other. The polarization (E_pol), dispersion (E_dis) and exchange-repulsion (E_rep) energies, which were obtained from 12 different models of the CrystalExplorer (CE) program, of the reference molecule of the complex was found as -0.2 kJ/mol, -3.2 kJ/mol and 0, respectively. On the other hand, the electrostatic (E_ele) energies of the molecule were obtained among 3.4 kJ/mol and 2.2 kJ/mol by using 12 different model of the program.

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Fare Kemik İliği Hücrelerinde Siklofosfamid Tarafından İndüklenen Genotoksisiteye Karşı Tarhun (*Artemisia dracunculus* L.) Yaprak Ekstraktının Olası Koruyucu Etkisinin Mikronükleus Testi ile Belirlenmesi

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Araştırma Makalesi Research Article	Biyoloji Biology	Geliş Tarihi/Received 13.10.2020	Kabul Tarihi/Accepted 28.12.2020

Öz: Bu çalışma; siklofosfamid (CP) tarafından indüklenen genotoksisiteye karşı, tarhun (*Artemisia dracunculus* L.) yaprak ekstraktının olası koruyucu etkisinin belirlenmesi amacıyla yapılmıştır. Çalışmada her bir grupta 8 hayvan olacak şekilde toplam 6 grup oluşturuldu. Bu gruplar; I. grup: negatif kontrol grubu, II. grup: pozitif kontrol grubu (50 mg/kg, i.p. CP), III. grup: 75 mg/kg tarhun yaprak ekstraktı grubu, IV. grup: 150 mg/kg tarhun yaprak ekstraktı grubu, V. grup: 75 mg/kg tarhun yaprak ekstraktı + CP (50 mg/kg, i.p.) grubu, VI. grup: 150 mg/kg tarhun yaprak ekstraktı + CP (50 mg/kg, i.p.) grubu şeklindedir. 14 gün boyunca tarhun yaprak ekstraktı oral gavaj yol ile farelere verildi. Fareler disloke edilmeden 24 saat önce (15. gün) intraperitonal olarak siklofosfamid enjekte edildi. Disloke edilen farelerin kemik iliği hücrelerinde mikronükleus testi yapılarak, mikronükleus oranları tayin edildi. Çalışma sonunda; mikronükleus sayılarının istatistiksel olarak değerlendirilmesi neticesinde negatif kontrol grubu ile kıyaslama yapıldığında, CP uygulanan grubun MN sayısının negatif kontrol grubuna göre artış gösterdiği belirlenmiş ($p<0.001$), CP ile birlikte 75 mg/kg ve 150 mg/kg dozlarında AD uygulamasına bağlı olarak ise, MN sayılarında CP grubuna göre oldukça önemli düzeyde azalma tespit edilmiştir ($p<0.001$). PCE/NCE oranları açısından değerlendirme yapıldığında ise, yine negatif kontrol grubuna göre CP grubu değerlerinin oldukça düşük olduğu ($p<0.001$), CP ile birlikte 75 mg/kg AD uygulandığında ($p<0.005$) ve 150 mg/kg AD uygulandığında ($p<0.001$) CP grubuna göre PCE/NCE oranlarının azaldığı belirlenmiştir. Sonuç olarak; *Artemisia dracunculus* L. yaprak ektraktının 75 mg/kg ve 150 mg/kg dozlarının fare kemik iliği hücrelerinde siklofosfamidin neden olduğu mikronükleus artışını azaltarak koruyucu etki gösterdiği belirlendi.

Anahtar Kelimeler: *Artemisia dracunculus* L., *Mus musculus*, Siklofosfamid, Mikronükleus Testi

Determination Of The Possible Protective Effect Of Tarhun (*Artemisia dracunculus* L.) Leaf Extract In The Mouse Bone Marrow Cells Against Cyclophosphamid By Micronucleus Test

Abstract: This work; had been carried out by aiming the determination of the possible protective effect of the tarragon (*Artemisia dracunculus* L.) leaf extract against genotoxicity of cyclophosphamide (CP), by micronucleus test. In the study, 6 test groups were formed and each group was involved 8 animals. These groups were named as: I. group is negative control group, II. group is positive control group which is formed from 50 mg/kg, i.p. CP, III. group denotes 75 mg/kg tarragon leaf extract group, IV. group denoted 150 mg/kg tarragon leaf extract group, V. group is formed from 75 mg/kg tarragon leaf extract + CP (50 mg/kg, i.p.) and VI. group is formed from 150 mg/kg

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tarragon leaf extract + CP (50 mg/kg, i.p.). The tarragon leaf extract was given to the mice via oral gavage during 14 days. Cyclophosphamide was injected intraperitoneally to the mice before 24 hours of the dislocation (day 15) of them. The micronucleus ratios were determined by the performing of the micronucleus tests to the bone marrow cells of dislocated mice.At the end of the study; when the statistical evaluation of micronucleus numbers were compared with the negative control group, it was determined that the MN numbers increased in the group which was exposed to CP ($p<0.001$) according to the negative control group. On the other hand, it was determined that the MN numbers of CP group were significantly decreased when the 75 mg/kg and 150 mg/kg doses AD in addition to the CP ($p<0.001$) were applied to the CP group.When an evaluation was carried out upon the PCE/NCE ratios, it was determined that values of the CP group ($p<0.001$) were less than values of the control group, on the other hand, when 75 mg/kg AD applied with CP ($p<0.005$) it was observed that the PCE/NCE ratios were decreased compared to the situation that applied 150 mg/kg AD to the CP group ($p<0.001$).As a result; it was determined that 75 mg/kg and 150 mg/kg doses of *Artemisia dracunculus* L. leaf extract showed protective effect in mouse bone marrow cells by leading to decrease of the increase in micronucleus which was caused by cyclophosphamide.

Keywords: *Artemisia dracunculus* L., *Mus musculus*, Cyclophosphamide, Micronucleus Test

1. GİRİŞ

Tarhun (*Artemisia dracunculus* L.) Asteraceae familyasına ait, otsu ve çok yıllık bir bitkidir. Doğrusal olarak büyüyen, demet halinde bulunan ve yukarı kısımları çatallara ayrılan bitki, kuvvetli köklere ve kısa yan köklere sahiptir. Bitkinin gövdesi ise yuvarlak hatlıdır ve 60-120 cm uzunluğa kadar erişebilmektedir. Tarhun yapraklarının alt kısmında bulunan yağ bezeleri sayesinde, bitki aromatik bir özellik kazanmaktadır. Tarhunun ana yurdu Sibirya'dır. Sonrasında Asya, Avrupa ve Kuzey Amerika'ya yayılmıştır. Türkiye'de de özellikle Bayburt, Erzurum, Gaziantep ve Şanlıurfa'da tarhun yetiştirilmektedir. *Artemisia dracunculus* L. Fransız ve Rus tarhunu olmak üzere iki varyeteye sahiptir (Ceylan, 1989; Fernandez-Lizarazo, Mosquera-Vasquez, Chaves and Sarmiento, 2011; Froushani, Zarei, Ghaleh and Motlagh, 2016; İlisulu, 1992).

Tarhun yüzyıllar boyunca insanlar tarafından; iştah, sindirim, romatizma, kan dolaşımı, uykusuzluk, cilt iltihapları, hayvan ısırıkları, sıtma, ülser, iskorbüt, gece körlüğü, epilepsi, diyabet ve kardiovasküler hastalıklar gibi çok çeşitli hastalıkların tedavisi için kullanılmıştır. Ayrıca Fransız tarhunundan elde edilen uçucu yağ; antioksidan, antifungal, antikanser ve antibakteriyel etkiler göstermektedir (Asımgil, 1997; Azırak, 2007; Gholivand, Yamini and Dayeni, 2014; Güner, Özhatay, Ekim and Başer, 2000). Hastalıklara karşı tedavinin dışında, tarhun (*Artemisia dracunculus* L.) mutfak dünyasında da kendine geniş bir kullanım alanı bulmuştur. Aromasından ötürü içeceklerde, yemeklerde, konservelerde, likör yapımında, şekerlemelerde ve peynir yapımında kullanılmaktadır. Tarhun aroması mutfak dışında parfüm yapımında da yer almıştır (Chaleshtori, Rokni, Razavilar and Kopaei, 2013; Graven, Webber, Venter and Gardner, 1990; Kırtunç, 2002).

Bitkilerin ekstrakt haline getirilerek şifa amacı ile kullanılması, Çin'de MÖ 2700 yıllarına kadar dayanmaktadır. Hitit dönemi tıbbi tabletleri üzerinde bulunan reçetelerdeki bitki isimleri ise Anadolu insanının uzun yıllardır yabani bitkilerden şifa aradığının bir göstergesidir (Toroğlu ve Çenet, 2006; Yiğit ve Benli, 2005). Uzun yıllardır yapılan bilimsel çalışmalarda tarhun bitkisinden elde edilen yağlardaki mevcut bileşenler araştırılmıştır. Bu bileşenler bölgelerin ekolojik durumuna göre değişiklik göstermektedir. Farklı coğrafik bölgelerde yapılan çalışmalarda tarhun bitkisinde en sık rastlanan bileşenler; estragol, elemisin, metil öjenol ve terpinolen olmuştur (Lamian, Badi, Mehrafarin and Seifsahandi, 2017; Tak, Mohiuddin, Ganai, Chishti, Ahmad and Dar, 2014; Wierdak and Zaviślak, 2014).

Siklofosfamid, azotlu hardal (nitrojen mustard) türü olan alkilleyici özelliğe sahip ilaçlardan biridir. Siklofosfamidin aktif metaboliti FAM (fosforamid mustard)'dır ve siklofosfamid karaciğerde bu yapıya dönüşerek etkili hale gelmektedir. Dönüşüm sonrası aktiflik kazanan siklofosfamid, DNA'ya bağlanıp alkilleşerek, DNA transkripsiyonu ve DNA replikasyonunda hasar meydana getirmektedir (McEvoy, 2004; Karaboğa, 2018). Siklofosfamid uygulaması ile ikincil kanserler arasında bir bağ olduğu ortaya çıkarıldığı için siklofosfamid, insan karsinojeni sınıfına dahil edilmekte ve pro-ilaç olarak adlandırılmaktadır (Doğan, 2008).

Canlılar üzerinde olumsuz etki meydana getiren ve bu etki sonrasında mutasyona neden olan fiziksel ve kimyasal maddelerin belirlenmesi amacı ile birçok test sistemi geliştirilmiştir. Günümüzde oldukça fazla kullanılmakta olan bu test yöntemleri, bileşiklerin genotoksik etkisinin tespitini yapmaktadır. En çok da kimyasallar, çevre kirliliği ve ilaçlar ile oluşan zehirlerde bu test yöntemleri tercih edilmektedir. Fakat yapılan bu testler çoğunlukla yeterli olmadığı için ek olarak akut ve kronik toksisite yöntemleri, antioksidan aktivite ve alerjik testlere de başvurulabilir (Klug and Cummings, 2002).

En yaygın olarak kullanılan standart in vitro ve in vivo mutajenite testleri; ames testi, comet testi, kromozom anormallikleri testi, kardeş kromatit değişimi testi ve mikronükleus testidir. Mikronükleus testi; *in vivo* ve *in vitro* şeklinde değerlendirilebilen, birtakım kimyasal ve fiziksel maddelerin neden olduğu klastojenik ve anöjenik etkilerin tespitinde kullanılan, genotoksisite test yöntemlerinden biridir. MN test yöntemi 1950'lerde ilk kez bitki hücrelerinde meydana gelen kromozom anomalilerinin tespitinde kullanılmıştır (Özkara ve Akyıl, 2015; Önen, Kılıçle and Doğan, 2017).

Bu çalışma ile, hücresel proteinlerin işlevini değiştirip DNA'ya hasar vererek alkilleyici ajan görevi gören siklofosfamidinin, fare kemik iliği hücrelerinde meydana getirdiği

genotoksiteteye karşı *Artemisia dracunculus* L. yaprak ekstraktının koruyucu etkisinin mikronükleus testi ile araştırılması amaçlanmıştır.

2. MATERYAL VE METOT

2.1.Hayvan Materyali

*Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından 2019/133 sayılı izin ile çalışma onayı alındı.

Çalışma esnasında mikronükleus analizinin yapılması amacıyla 12 haftalık, ağırlıkları 32-47 gram arasında değişen, 48 adet *Mus musculus* cinsi albino fare kullanıldı. Fareler standart hayvan koşullarına uygun bir şekilde, 121°C'de otoklove edilebilen ve polikarbon malzemelerden üretilmiş olan kafeslerde 8'li gruplar halinde konumlandırıldı. Su ve yem ihtiyaçlarının ad libitum olması sağlanarak, fareler distile su ve normal fare yemi ile beslendi. Fareler 20 ± 2 °C sıcaklığa ve % 50 bağıl neme sahip, 12 saat aydınlık ve 12 saat karanlık ışık periyodu olan laboratuvar ortam ve koşullarında barındırıldı. Farelerin günlük ağırlıklarına göre çalışmada kullanılacak maddelerin dozları belirlendi. Maddeler distile su ile çözülüp, oral gavaj ve intraperitonal (i.p.) yöntemler ile farelere verildi.

2.2. *Artemisia dracunculus* L. Yaprak Ekstraktı

Çalışmada kullanılan tarhun (*Artemisia dracunculus* L.) Bayburt ili Yerlice Köyü'nden haziran ayında toplandı. Bitki mevsiminde ve taze olarak laboratuvar ortamına ulaştırıldı. Kafkas Üniversitesi Botanik Anabilim Dalı uzmanları tarafından teşhis edildi. Bitkinin yaprak kısımları alınarak güneş görmeyen ve hava sirkülasyonuna sahip olan alanda kurutuldu. Tamamen kurutulan bitki yaprakları öğütücü yardımı ile öğütüldü. Ekstraksiyon çözücüsü ile yıkanan soxhlet cihazı kartuşunun içerisine 40 gram öğütülmüş yaprak örneklerinden alınarak konuldu. Tarhun yaprak örneğini içeren kartuş 500 ml'lik soxhlet ekstraktörü içerisine yerleştirildi. Kaynama balonuna ekstraksiyon çözücüsü olarak 650 ml etanol konuldu. Yaklaşık olarak 8 saat süre ile çözücü berrak hale gelinceye kadar 7-10 kez sifon edildikten sonra sıvı ekstrakt elde edildi. Sıvı ekstrakt içerisinde bulunan partiküller, mavi band süzgeç kağıdı kullanılarak uzaklaştırıldı. Partiküllerinden arınan ekstrakt örneğindeki çözücüler, 35-45 °C'de rotary evaporatör ile uçuruldu. Balon içerisinde geriye kalan tarhun yaprak ekstraktı, desikatörde 12 saat süre ile bekletildi. Çözücüsünden tamamen ayrılmış olan tarhun yaprak ekstraktı numune ekstrakt kutusuna konularak, yapılacak olan çalışma için +4 °C'de muhafaza edildi (Wang and Weller, 2006).

2.3. Deney Grupları

Fareler her grupta 8 adet olacak şekilde 6 gruba ayrıldı. Bunlar;

1. Grup: (n:8) Negatif kontrol. Diğer gruptaki fareler ile aynı stresi yaşamaları için, bu gruptaki farelere 14 gün boyunca distile su oral gavaj yol ile verildi. 15. gün eter anestezisi altında servikal dislokasyon yapıldı.

2. Grup: (n:8) 50 mg/kg siklofosfamid (CP). Bu gruptaki farelere 14 gün boyunca distile su oral gavaj yol ile verildi. Farelere 50 mg/kg siklofosfamid 14. gün intraperitonal yol ile verildi. 15. gün eter anestezisi altında servikal dislokasyon yapıldı (CP'nin 24 saatlik etkisine bakıldı).

3. Grup: (n:8) 75 mg/kg tarhun yaprak ekstraktı. Bu gruptaki farelere 75 mg/kg tarhun (*Artemisia dracunculus* L.) yaprak ekstraktı 14 gün boyunca oral gavaj yol ile verildi. 15. gün eter anestezisi altında servikal dislokasyon yapıldı.

4. Grup: (n:8) 150 mg/kg tarhun yaprak ekstraktı. Bu gruptaki farelere 150 mg/kg tarhun (*Artemisia dracunculus* L.) yaprak ekstraktı 14 gün boyunca oral gavaj yol ile verildi. 15. gün eter anestezisi altında servikal dislokasyon yapıldı.

5. Grup: (n:8) 75 mg/kg tarhun yaprak ekstraktı + CP. Bu gruptaki farelere 75 mg/kg tarhun (*Artemisia dracunculus* L.) yaprak ekstraktı 14 gün boyunca oral gavaj yol ile verildi. 14. günün sonunda 50 mg/kg dozda CP intraperitonal yol ile verildi. 15. gün eter anestezisi altında servikal dislokasyon yapıldı.

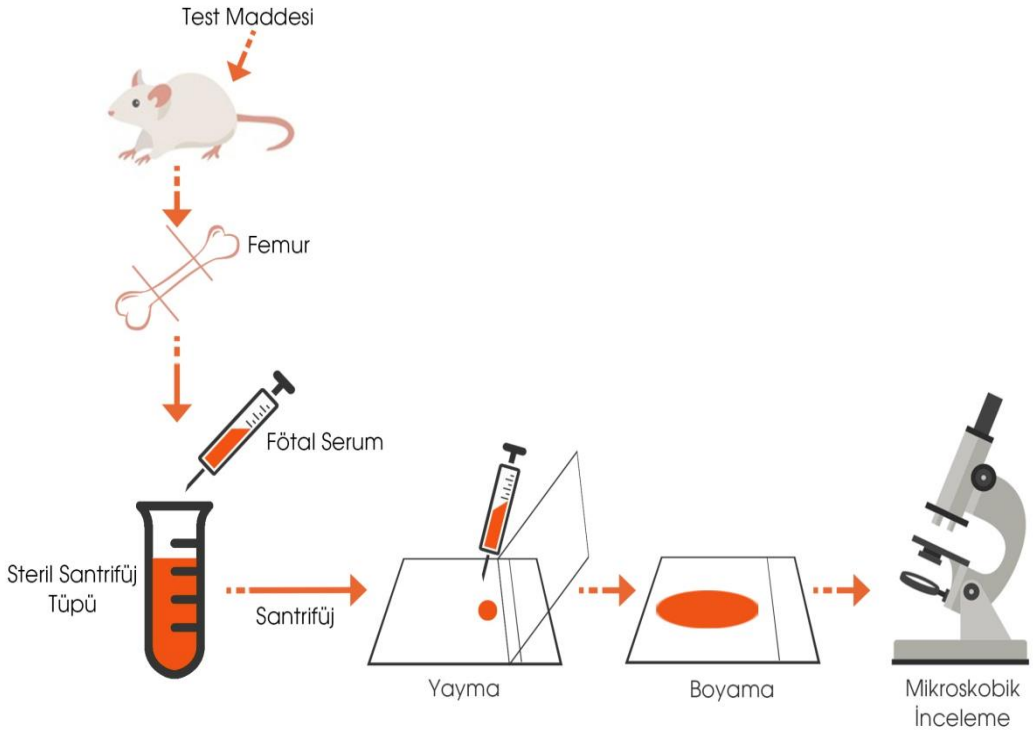
6. Grup: (n:8) 150 mg/kg tarhun yaprak ekstraktı + CP. Bu gruptaki farelere 150 mg/kg tarhun (*Artemisia dracunculus* L.) yaprak ekstraktı oral gavaj yol ile 14 gün verildi. 14. günün sonunda 50 mg/kg dozda CP intraperitonal yol ile verildi. 15. gün eter anestezisi altında servikal dislokasyon yapıldı.

2.4. Mikronükleus testi

Yapılan çalışmada mikronükleus testi için fare kemik iliği kullanıldı. İncelemede kullanılmak üzere hazırlanan preparatlar, ilk kez Schmid tarafından 1975 yılında geliştirilen kemik iliği preparat yöntemi ışığında yapıldı (Şekil 1).

Öncelikle ilik için farelerden çıkarılan femur kemiği kaslarından tamamen temizlendi ve iki ucundan bistüri yardımı ile kesildi. Enjektör ile kemik içerisindeki ilik, 3 ml fötal dana serumu içeren santrifüj tüpüne aktarıldı. Tüpler 5 dakika boyunca 2000 rpm'de santrifüj edildi ve sonrasında süpernatantları pasteur pipetle alınarak atıldı. Sonrasında tüp içerisine bir damla fötal dana serumu eklendi ve hücreler süspanse edildi. Tüplerden birer damlalık örnekler alındı ve temiz lamlara yayıldı. Yayma işleminden sonra havada kurutulan preparatlar, 10 dakika

boyunca metil alkolde fikse edildi. Preparatlar fikse edildikten sonra, ilk olarak % 0.25'lik May Grunwald boyası ile 5 dakika boyunca boyandı ve saf su ile yıkandı. Sonrasında preparatlar % 0.125'lik May Grunwald boyası ile tekrar 5 dakika boyunca boyanıp, saf su ile yıkandı. Son olarak % 20'lik Giemsa boyası ile 30 dakika boyunca boyanan preparatlar, saf su ile yıkanıp oda sıcaklığında kurumaya bırakıldı. İmmersiyon objektifinde (1000'lik büyütme) her deney grubundan gelişigüzel 2000 adet polikromatik eritrosit (PCE) hücre sayıldı. Mikronükleuslu polikromatik eritrosit (MNPCE) içeren hücreler sayılarak, MNPCE oranları belirlendi. Bunlara ek olarak 1000 adet normokromatik eritrosit (NCE) ve PCE sayılarak, PCE/NCE yüzdelik oranları tespit edildi (Schmid, 1975; Doğan, 2019; Aksu, Doğan, Gül and Kanıcı, 2013).



Şekil 1. *İn vivo* Mikronükleus Test Protokolü

2.5. Tarhun Bitkisinin Uçucu Yağ Analizi

Tarhun yaprak ekstraktının uçucu yağ bileşenlerinin ve bu bileşenlerin bağıl yüzdelerinin tayin edilmesi için, kurutulmuş 40 gr tarhun bitkisi Anadolu Üniversitesi Bitki, İlaç ve Bilimsel Araştırmalar Uygulama ve Araştırma Merkezi'ne gönderildi. Merkezde Gaz Kromatografisi (GC) yöntemi ile yağ analizi yapıldı. Yapılan analiz sonucunda elde edilen sonuçlar Tablo 1'de gösterilmiştir.

Yöntem

Örnek maddenin uçucu yağının bileşenlerinin tanımlanması için Gaz Kromatografisi/Kütle Spektrometresi, bağıl yüzdelerinin belirlenmesi için ise Gaz Kromatografisi yöntemi kullanılmıştır.

Numune Hazırlama

Hekzan ile hazırlanan (% 10 h/h) örnek 40:1 split oranı ile 1 µL olarak sisteme enjekte edilmiştir.

Gaz Kromatografisi (GC) Şartları

Sistem: Agilent 7890B GC System
Kolon: Agilent HP-Innowax (60 m × 0.25 mm iç çap × 0.25 µm film kalınlığı)
Dedektör: Alev İyonlaşma Dedektörü (FID)
Enjeksiyon Sıcaklığı: 250°C
Dedektör Sıcaklığı: 250°C
Sıcaklık Programı: 60°C (10 dk), 4°C/dk. 220°C (10 dk) 1°C/dk 240°C Toplam 80 dk
Taşıyıcı Gaz: Helyum (0.7 mL/dk)

Gaz Kromatografisi/Kütle Spektrometresi (GC/MS) Şartları

Sistem: Agilent 7890B GC 5977B Mass Selective Dedector System
Kolon: Agilent HP-Innowax (60 m, 0.25 mm iç çap, 0.25 µm film kalınlığı)
Enjeksiyon Sıcaklığı: 250°C
İyon Kaynağı Sıcaklığı: 230°C
İyonizasyon Modu: EI
Elektron Enerjisi: 70 ev
Kütle Aralığı: 35- 450 m/z
Sıcaklık Programı: 60°C (10 dk), 4°C/dk. 220°C (10 dk) 1°C/dk 240°C, Toplam 80 dk
Taşıyıcı gaz: Helyum (0.7 mL/dk)
Tanımlamalar: Wiley 9-Nist 11 Mass Spectral Database (Özek, 2020)

Tablo 1: *Artemisia dracunculus* Uçucu Yağının Bileşimi

No	**Bileşik***	**Bağıl yüzde (%)**
1	Estragol	67,7
2	(Z)- β-Osimen	5,5
3	(E)- β-Osimen	5,1
4	Spatulenol	4,5
5	Limonen	3,9
6	Metil öjenol	2,0
7	α-Pinen	1,4
(* ≥ % 1)**Toplam**		**90.1**

2.6. İstatistik Analiz

Çalışmadan elde edilen verilerin istatistiksel analizleri için SPSS 22 paket programı kullanıldı. Kontrol ve deney grupları arasındaki farklılığın belirlenmesi için, tek yönlü varyans analizi (One-Way ANOVA) yapıldı ve $p<0.05$ istatistiksel olarak önemli kabul edildi

3. BULGULAR

3.1. Fare Kemik İliği Hücrelerinde Kontrol ve Deney Gruplarının Mikronükleus Frekansları

Mikronükleus testi için gruplardaki her bir fare için 3 adet kemik iliği preparatı hazırlandı. Mikronükleus frekansının belirlenmesi adına her bir hayvandan 2000 adet PCE (Polikromatik Eritrosit) sayımı yapıldı (Tablo 2).

Tablo 2. Kontrol ve Deney Gruplarının Mikronükleus Test Sonuçları

Gruplar	**Toplam PCE**	**MNPCE**	**MNPCE (%)**	**Grup Ortalaması**	**Toplam Eritrosit (PCE+ NCE)**	**PCE Sayısı**	**NCE Sayısı**	**PCE/ NCE Oranı**
Negatif Kontrol	16000	312	1,950	39,00	8000	5533	2467	2,24
50 mg/kg CP	16000	913	5,70	114,125	8000	3912	4088	0,95
75 mg/kg AD	16000	329	2,056	41,12	8000	5382	2618	2,05
150mg/kg AD	16000	338	2,106	42,25	8000	5422	2578	2,09
75 mg/kg AD + 50 mg/kg CP	16000	695	4,34	86,875	8000	4099	3901	1,04
150 mg/kg AD + 50 mg/kg CP	16000	617	3,85	77,125	8000	4719	3281	1,43

***PCE:** Polikromatik Eritrosit, **NCE:** Normokromatik Eritrosit, **MNPCE:** Mikronükleuslu Polikromatik Eritrosit.

PCE/NCE oranları açısından değerlendirme yapıldığında; negatif kontrol grubuna göre CP grubu değerlerinin oldukça düşük olduğu ($p<0.001$), CP ile birlikte 75 mg/kg AD yaprak ekstraktı uygulandığında ($p<0.0.05$) ve 150 mg/kg AD yaprak ekstraktı uygulandığında ($p<0.001$), CP grubuna göre PCE/NCE oranlarının azaldığı belirlenmiştir.

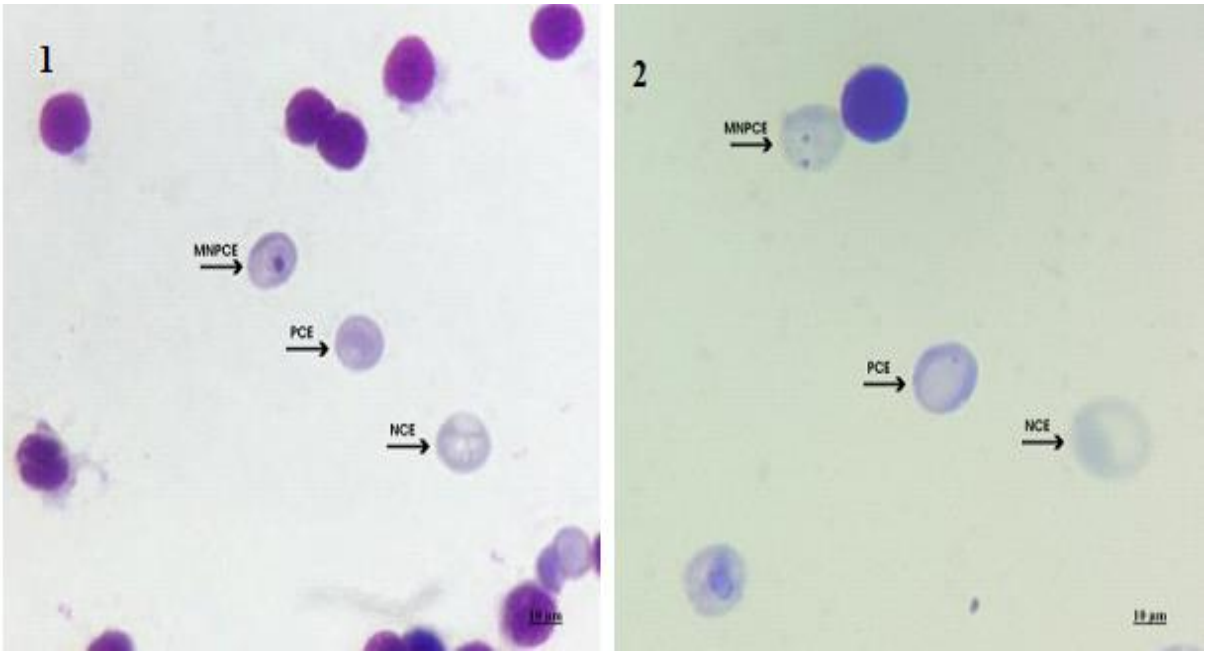
Mikronükleus sayılarının istatistiksel olarak değerlendirilmesi neticesinde; negatif kontrol grubu ile kıyaslama yapıldığında, CP uygulanan grubun MN sayısının negatif kontrol grubuna göre artış gösterdiği belirlenmiş ($p<0.001$), CP ile birlikte 75 mg/kg ve 150 mg/kg dozlarında AD yaprak ekstraktı uygulamasına bağlı olarak ise MN sayılarında CP grubuna göre oldukça önemli düzeyde azalma tespit edilmiştir ($p<0.001$).

Tablo 3. Kontrol ve Deney Gruplarına Ait Mikronükleus ve PCE/NCE Oranlarının İstatistiki Sonuçları

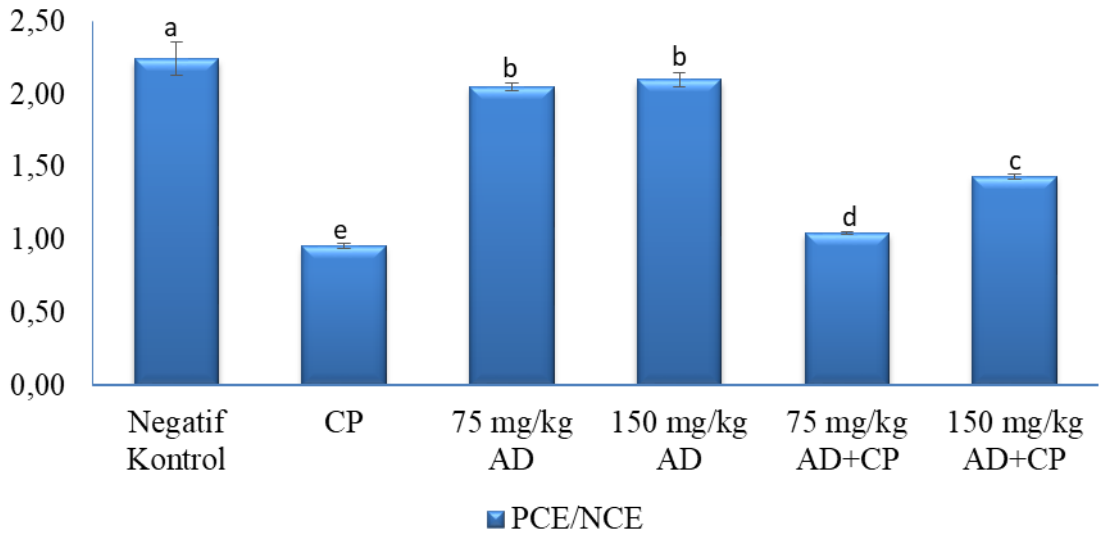
Gruplar	**NK**	**50 mg/kg CP**	**75 mg/kg AD**	**150 mg/kg AD**	**75 mg/kg AD+ 50 mg/kg CP**	**150mg/kg AD+ 50 mg/kg CP**	**P Değeri**
PCE/ NCE Oranları	2,24 ±0,11[a]	0,95 ±0,02[e]	2,05 ±0,03[b]	2,10 ±0,05[b]	1,05 ±0,01[d]	1,43 ±0,02[c]	0.001
MNPCE Oranları	39,00 ±1,31[e]	114,13±2,47[a]	41,13 ±1,55[de]	42,25 ±2,25[d]	86,88 ±1,81[b]	77,13 ±2,03[c]	0.001

* Aynı satırdaki farklı harfler istatistiksel önemliliği ifade etmektedir.

Aşağıdaki resimlerde PCE, NCE ve MNPCE'ler gösterilmiştir (Resim 1).

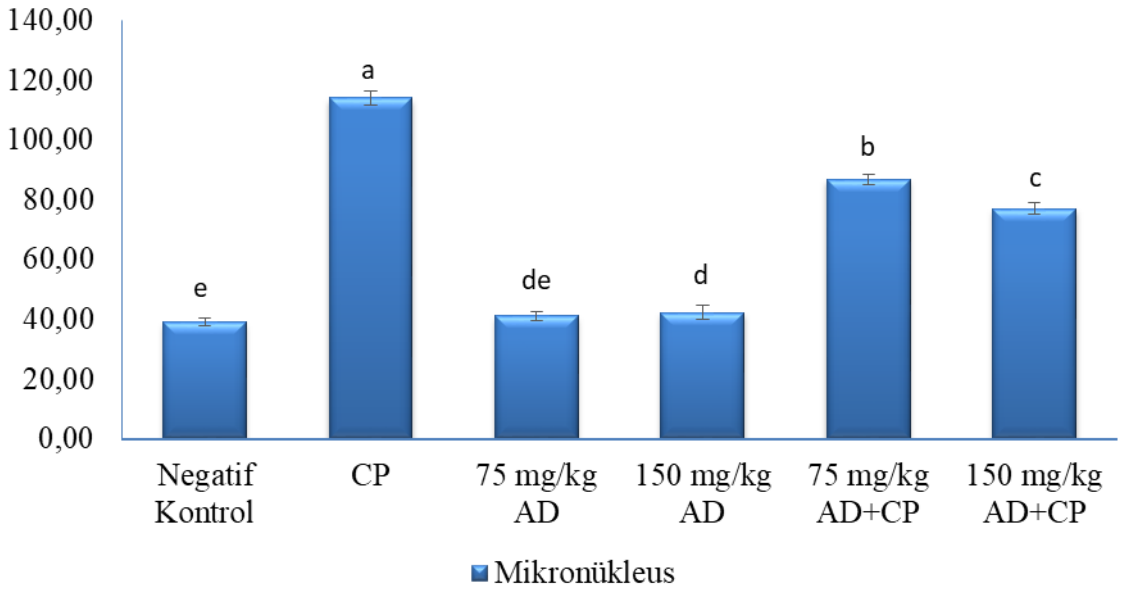


Resim 1: Deney Grubu Farelerinin Kemik İliği Hücrelerinde MNPCE, PCE ve NCE'lerin Mikroskobik Görüntüsü (×1000) 2: Deney Grubu Farelerinin Kemik İliği Hücrelerinde 2 Mikronükleuslu MNPCE, PCE ve NCE'lerin Mikroskobik Görüntüsü (×1000)



$^{a\text{-}e}$, $^{e\text{-}c}$ $p<0.001$, $^{e\text{-}d}$ $p<0.05$

Şekil 2. Kontrol Grubu ve Uygulama Gruplarına ait PCE/NCE Oranları



$^{a\text{-}e}$, $^{a\text{-}b}$, $^{a\text{-}c}$ $p<0.001$

Şekil 3. Kontrol Grubu ve Uygulama Gruplarına ait MNPCE Oranları

4. TARTIŞMA VE SONUÇ

Son zamanlarda fiziksel ve kimyasal maddelerin DNA'da meydana getirdiği genotoksik etkileri en az seviyede tutabilmek için birçok çalışma yapılmıştır. Yapılan bu çalışmalar ile doğal bitkisel ürünlerin antigenotoksik etkilerinin araştırılması, doğal antioksidanların önemini arttırmıştır (Ferguson, Bronzetti and De-Flora, 2005).

İnsanoğlu, maruz kaldıkları mutajenlerin negatif etkilerine karşı, vücutlarında var olan doğal antioksidanlar ile korunmaktadırlar. Yaşın ilerlemesine bağlı olarak vücudumuzda var olan doğal antioksidanların üretiminde yavaşlama meydana gelmektedir. Bu sebepten ötürü insanlar dışarıdan antioksidan desteğine ihtiyaç duyarlar. Çeşitli antigenotoksik bileşiklerin kullanımı, canlıların vücudunda oluşan ve genotoksik ajanların neden olduğu DNA hasarlarının etkilerini en aza indirgemek amacıyla arttırılmaktadır. Bu durumun, mutasyonların neden olduğu kanser ve bunun gibi hastalıklardan korunmak için önem taşıdığı bildirilmektedir. *Artemisia dracunculus* içeriğinde antioksidan aktivite özelliği olan bileşiklerin var olduğu, çeşitli *in vivo* çalışmalar ile belirlenmiştir. Polifenol olarak bilinen bu faydalı antioksidanlar, vücudumuzda bulunan serbest radikallere karşı güçlü bir savunma sistemidir (Garcia, Filippi, Mosesso, Calvani, Nicolai, Mosconi and Palitti, 2006; Taner, 2007; Madrigal-Bujaidar, Barriga, Cassani, Marquez and Revuelta, 1998; Malins, Johnson, Wheeler, Barker, Nayak, … Vinson, 2001).

Bu çalışmada bitkideki polifenolik içerikler belirlenmemiştir. Ancak bitkinin uçucu ve doymamış yağ asitleri yönünden analizi yapılmıştır. Doymamış olduklarından bu yağ asitlerinin diğer araştırmacılar tarafından tespit edilen polifenoller gibi antioksidan etkide rol oynayabileceği düşünülmektedir.

Yapılan bir çalışmada, CCl_4 (karbon tetraklorür) ile 60 adet Wistar albino ratta oluşturulan akut karaciğer hasarına karşı, tarhun yaprak ekstraktının koruyucu etkisi araştırılmıştır. 6 hafta boyunca gün aşırı gavaj yoluyla 250, 500, 750 mg/kg dozlarında AD yaprak ekstraktı ve intraperitonal yol ile zeytinyağı ile karıştırılmış CCl_4 (0.2 mg/kg) verilmiştir. Deney sonrasında ratların karaciğer dokuları histopatolojik açıdan incelenmiştir. Etanolik AD ekstraktı verilen bütün gruplarda, bitkinin histopatolojik ve biyokimyasal açıdan olumlu etki yarattığı, AD'nin ratların karaciğerinde CCl_4 etkisi ile oluşturulan hasara karşı koruyucu etki gösterdiği ve karaciğeri güçlendirdiği rapor edilmiştir (Gülpınar, 2012).

Zarezade ve arkadaşları, CCl_4 ile oluşturulan hepatotoksisiteye karşı, tarhun bitkisinin gövde ve yaprak kısımlarından elde edilen hidro-alkolik *Artemisia dracunculus* (HAAD) ekstraktının, ratlar üzerindekiantioksidan ve hepotoprotektif etkisini araştırmışlardır. 36 adet rata, 50, 100 ve 200 mg/kg HAAD 7 gün boyunca oral gavaj ile verilmiştir. Çalışmada yapılan FRAP (Fe3+ (ferik iyonu) indirgeme gücü), DPPH (Radikali giderme aktivitesi) ve ABTS (Radikal katyon yakalama aktivitesi) biyokimyasal testleri sonucunda; HAAD'in güçlü bir aktivite gösterdiği, oluşan hepatik hasara karşı koruyucu etki sergilediğini rapor etmişlerdir (Zarezade, Moludi, Mostafazadeh, Mohammadi and Veisi, 2018).

Hong ve Ying yaptıkları bir çalışmada; özofagus skuamöz hücre karsinomuna karşı, *Artemisia dracunculus* bitkisinin sürgün ve kök kısmından elde edilen ekstraktın ve bileşenlerinin antitümör etkisini araştırmışlardır. Mevcut karsinomun tedavisinde AD ekstraktı kullanılmıştır. Yapılan flow sitometri testi sonucunda; bitki ekstraktının özofagus hücre karsinomuna karşı güçlü şekilde hücre çoğalmasını önleyici aktiviteler gösterdiği görülmüştür. Ayrıca AD bitkisinden elde edilen sakuranetin ve 6-metoksikapillarisin bileşenlerinin, özofagus skuamöz hücrelerinde DNA hasarını indükleyerek güçlü antikanser etkiler sergilediği gözlenmiştir (Hong and Ying, 2015).

Modaresi ve arkadaşları fareler üzerinde yaptıkları bir çalışmada, farelerin hematolojik parametreleri üzerinde, hidro-alkolik AD yaprak ekstraktının yaratacağı etkileri araştırmışlardır. 5 gruba ayrılmış 40 fareye 20 gün boyunca gün aşırı sırasıyla 50, 100 ve 200 mg/kg dozunda hidro- alkolik AD yaprak ekstraktı verilmiştir. Deney sonunda yapılan ölçüm (RBC, WBC, nötrofil, monosit ve lenfosit) ve analizler (varyans test) sonucunda; kontrol gruplarına oranla tedavi gruplarında monosit, RBC ve WBC oranlarında kayda değer bir değişikliğin yaşanmadığı görülmüştür. 100 mg/kg ve 200 mg/kg tedavi gruplarında, kontrol gruplarına oranla lenfosit sayısında azalış yaşanırken, nötrofil sayısında anlamlı bir artış yaşanmıştır. Yapılan araştırma sonucunda, immünostimülatör bir ajan olarak tercih edilebilecek nötrofil üretiminin, etanolik AD yaprak ekstraktı sayesinde uyarıldığı sonucuna varılmıştır (Modaresi, Zarasvand and Madani, 2018).

Abraham, farelerde trans-anetol ve öjenolün antigenotoksik etkisini mikronükleus testi ile araştırmıştır. Genotoksin olarak farelere 40-400 mg/kg oranında trans-anethol, 50-500 mg/kg oranında öjenol oral gavaj yol ile verilmiştir. Genotoksin olarak verilen maddelerden biri de siklofosfamid olmuştur. CP farelere intraperitonal olarak verilmiştir. Yapılan mikronükleus analizi sonucunda, farelere uygulanan trans-anethol ve öjenolün siklofosfamidin neden olduğu genotoksisiteye karşı koruyucu etki gösterdiği rapor edilmiştir (Abraham, 2001).

Tüylü ve arkadaşlarının yaptıkları çalışmada; *Artemisia dracunculus* bitkisinden elde edilen esansiyel yağın, anti-mikrobiyal, genotoksik ve sitotoksik etkisi araştırılmıştır. Çalışmada; *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli* ve *Aspergillus niger* gibi toplam 28 adetmikroorganizma kullanılmıştır. Kirby ve Bauer disk difüzyon yöntemi ile *Artemisia dracunculus* esansiyel yağının belirtilen mikroorganizmalara karşı anti-mikrobiyal etki sergilediği görülmüştür. Ayrıca mikronükleus testi için iki sağlıklı insandan elde edilen periferik kan kullanılmıştır. Sonuç olarak bitki ekstraktının genotoksik etki yaratmadığı gözlemlenmiştir (Tüylü, Yılmaz ve Kıvanç, 2009).

Bolandian ve arkadaşları; 70 wistar ratında asetik asit tarafından indüklenen ülseratif kolite karşı tarhun sulu yaprak ekstraktının etkilerini araştırmışlardır. 10 gün boyunca ratlara mesalazine, asacol ve asacol + tarhun sulu yaprak esktraktı oral gavaj yol ile verilmiştir. Deneyde yapılan Elisa testiyle birlikte histopatolojik incelemeler sonucunda; kontrol grubuna göre, mesalazine ve asacol tedavi gruplarında histopatolojik hasar artmıştır. asacol + tarhun sulu yaprak ekstraktı verilen tedavi gruplarında ise oluşan histopatolojik hasarın önemli düzeyde azaldığı görülmüştür. Sonuç olarak tarhun sulu yaprak ekstraktının anti-kolit etki sergilediği ve kolit tedavisinde doğal bir ilaç olarak kullanılabileceği görülmüştür (Bolandian, Dorostkar, Shadbad and Ghaleh, 2019).

Reza ve arkadaşlarının yaptığı bir çalışmada; 48 wistar albino ratta tarhun sulu yaprak esktraktının, sulandırılmış fruktoza karşı antienflamatuar ve nosiseptif etkileri araştırılmıştır. Oluşturulan 1. gruba hiçbir madde verilmemiştir. 2. gruba 100 mg/kg oranında tarhun sulu yaprak ekstraktı, 3. gruba ise % 10 oranında sulandırılmış fruktoz ve 100 mg/kg oranında tarhun sulu yaprak ekstraktı verilmiştir. 4 haftanın sonunda; ratlarda oluşturulan insülin direncinin, tarhun sulu yaprak ekstaraktı tarafından önemli şekilde azaltıldığı gösterilmiştir (Reza, Hamideh and Zahra, 2015).

Benli ve arkadaşları, AD'nin aseton, kloroform ve farklı iki konsantrasyondaki metanol ekstresinin antimikrobiyal aktivitesi üzerinde çalışmışlardır. Bu ekstraktlar, disk difüzyonu yöntemi ile 9 bakteri ve 4 maya suşuna karşı test edilmiştir. Sonuç olarak; AD'nin, metanol ekstraktının, aseton ve kloroform ekstraktlarına oranla mikroorganizmalara karşı daha yüksek antimikrobiyal etki gösterdiği belirlenmiştir (Benli, Kaya and Yiğit, 2007).

Ribnicky ve arkadaşları, tarhun bitkisinin diyet takviyesi olarak kullanımının toksikolojik değerlendirmesi ile ilgili ratlar üzerinde çalışmalarda bulunmuşlardır. Yapılan çalışmalar ve kullanılan AD yaprak ekstrakt dozları; 14 gün tekrar doz oral toksisite çalışması (1000 mg/kg), 90 gün tekrar doz oral toksisite çalışması (10, 100 ve 1000 mg/kg), ames testi (5000 mg/kg) ve akut oral toksisite testi (5000 mg/kg) şeklindedir. Yapılan çalışmalarda tarhun bitkisinin ratlar üzerinde mutajenik etki yaratmadığı, bitkinin kullanımının non-toksik ve güvenilir olduğu tespit edilmiştir (Ribnicky, Poulev, O'Neal, Wnorowski, Molek, Jager and Raskin, 2004).

Halk arasında hem tıbbi amaçlı hem de tüketime yönelik olarak kullanılan *Artemisia dracunculus* L.'nin antigenotoksik, antifungal, antioksidan, antitümoral vb. özellikleri birçok *in vivo* ve *in vitro* çalışma ile bildirilmiştir. Bu çalışma sonuçları, daha önce yapılan diğer çalışma sonuçları ile benzerlik göstermektedir. Bu araştırma sonucuna göre; tarhun yaprak ekstraktının uygulanan dozlarının (75-150 mg/kg) genotoksik etkili olmadığı ve siklofosfamid

tarafından indüklenen mikronükleus artışını azalttığı söylenebilir. Kullanılan bitki ekstraktının ortaya çıkardığı antigenotoksik etkilerin tam anlamıyla belirlenebilmesi ve antigenotoksik etkilerin hangi maddelerden kaynaklandığının tespit edilebilmesi için, bitki bileşenlerinin yapısındaki kimyasalların izole edilmesi gerekmektedir. Tarhun bitkisinden elde edilen ektrakt içerisinde tespit edilecek kimyasal bileşiklerin etkilerinin sitogenetik analiz ve ileri moleküler testler ile tespit edilmesinin, konunun anlaşılmasına fayda sağlayacağı ileri sürülebilir.

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Assessment of Mass Attenuation Coefficient, Effective Atomic Number and Electron Density of Some Aluminum Alloys

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Abstract

Aluminum alloys have numerous application fields in today's technology due to their excellent mechanical features, high electrical and thermal conductivity, magnificent corrosion resistance, good weldability, good formability and similar properties. In the present study, we investigated the mass attenuation coefficient (μ_m), effective atomic number (Z_{eff}) and effective electron density (N_e) of four different type commercially available aluminum alloys. For his purpose, μ_m, Z_{eff}, and N_e values of 5083, 5754, 6061 and 6082 coded aluminum alloys were determined by employing NaI(Tl) gamma ray spectrometry at 661.66,1173.23 and 1332.48 keV gamma ray energies obtained from ^{137}Cs and ^{60}Co radioactive sources. Also these parameters theoretically determined using PhyX-PSD computer program at the photon energies of 1 keV–1 GeV and compared with the experimental results. The variation of μ_m, Z_{eff}, and N_e with incident photon energy presented graphically. From the obtained results it might be concluded that the μ_m, Z_{eff}, and N_e values for studied alloy samples depend on the incident photon energy and elemental composition of alloys. In addition, it was observed from the theoretical and experimental results that aluminum alloys under study have almost the same gamma ray attenuation capacity.

Keywords: Gamma ray, Mass attenuation coefficient, Effective atomic number, Electron density

1. INTRODUCTION

Aluminum and its alloys have been charmed interest of many researchers, engineers and designers because of their high strength to weight ratio superior to steel and corrosion endurance. Aluminum alloys are created by adding copper, zinc, silicon, magnesium, manganese, iron, nickel, titanium and similar elements. The elements added to aluminum improve the properties of the material and make it superior to other metals. These alloys are widely used in many fields in today's technology such as automotive, aerospace, military and also nuclear reactors as the tank material for the TRIGA Mark Reactors as they are light and resistant to high temperatures and some chemical effects (Ozturk, Sisman, Toros, Kilic and Picu, 2016; Yıldırım, Tugrul, Buyuk and Demir, 2010;). Among the aluminum alloys, 6XXX and 5XXX series alloys are widely used in many application areas where good weldability and excellent corrosion resistance are needed. Magnesium is the major alloying element in the 5xxx

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series alloys and the use of magnesium creates a medium-to-high-strength workhardenable alloy. In the 6xxx series, magnesium and silicon are used together as basic alloying elements. These alloys become heat treatable alloys with the use of magnesium and silicon in the proportions required to form Mg_2Si. Due to the advantages like good extrudability, medium strength, formability, weldability, corrosion resistance and low cost, the application range of aluminum alloys is increasing day by day (Lee, Saito, Sakai and Utsunomiya, 2002). Various studies have been conducted to investigate the mechanical and physical properties of aluminum alloys for potential uses (Ozturk et al. 2010; Lee et al., 2002; Fuller, Krause and Dunand, 2002; El-Rayes and El-Danafa 2012).

Recently with the increase in the use of radiation in various applications in daily life, many researchers have focused on examining the gamma or X ray attenuation properties of many materials to reduce or retain X or gamma rays. In the literature, several experimental or computational studies have been extensively carried out to estimate X or gamma ray attenuation behaviors of diverse materials such as concrete (Akkurt, Basyigit, Kilincarslan and Mavi, 2005; Singh and Badiger 2014; Oto, Gur, Kavaz, Cakir and Yaltay, 2016), glass system (Mostafa, Issa and Sayyed, 2017; Kurudirek, Büyükyıldız and Özdemir, 2010; Sayyed, Kaky, Şakar, Akbaba, Taki and Agar, 2019a) stainless steel (Akkurt 2009; Singh, Medhat and Shirmardi, 2015; Alim, Şakar, Baltakesmez, Han, Sayyed and Demir, 2019) various ores (Oto, Yıldız, Akdemir and Kavaz, 2015) and like.

The mass attenuation coefficient (μ_m), effective atomic number (Z_{eff}) and effective electron density (N_e) are parameters of great importance to characterize the penetration of X or gamma rays in a material. The proper information of these radiation attenuation parameters in several materials is very helpful in medical, technological, agricultural, nuclear, space exploration and engineering applications (Sharma, Sharma, Kaur, Singh, Sharma and Singh, 2017). μ_m is defined as the probability of all interactions that occur between incident photons and the matter of the unit mass per unit area and depends on the incident photon energy and the chemical composition of the absorbing material. (Sharma, Sharma, Singh and Singh, 2012). Z_{eff} and N_e are parameters required to express the atomic and electron numbers of composite materials consisting of many elements such as alloys as a single number at a given energy value, and these parameters depend on the incident photon energy and the elemental diversity in the composite material (Şakar, Büyükyıldız, Alım, Şakar and Kurudirek, 2019).

Many studies have been extensively carried out in recent years for estimating radiation attenuation behaviors of various alloys using experimental techniques or computational methods. Han and Demir (2009) experimentally measured μ_m, Z_{eff} and N_e values for Ti, Ni

alloys at 22.1, 25.0, 59.5 and 88.0 keV photon energies. They also investigated variations of these parameters with photon energy. Şakar et al., (2019) investigated radiation attenuation behaviors of some leaded brass alloys. They experimentally determined μm, Z_{eff}, N_{eff} and other shielding parameters of leaded brasses at 53, 276, 302, 356 and 383 keV photon energies and compared with the theoretical values. Akman et al. (2019) studied gamma ray attenuation performance of some ternary alloys consist of Cr, Fe and Ni elements in the energy range of 81 keV-1333 keV. Singh et al. (2018) reported some gamma ray attenuation parameters such as μ_m, Z_{eff} and N_e for some xPb-(1-x)Cu Binary alloys at 511, 662 and 835 keV photon energies. Kurudirek et al. (2010) carried out an extensive study to determine effective atomic numbers of diverse alloys in the energy range of 1keV to 100GeV using WinXCom program. Singh et al. (2015) computed μ_m, Z_{eff} values for steel alloys using Geant4 and MCNP codes in the energy range 279.1–1332keV.Then they compared simulated results with theoretical and experimental data.

The primary purpose of this research is to estimate variation of μ_m, Z_{eff}, and N_e values of four different type aluminum alloys with photon energies. For this purpose, μ_m, Z_{eff}, and N_{eff} values for 5083, 5754, 6061 and 6082 coded aluminum alloys experimentally measured at gamma ray energies of 661.66, 1173.23 and 1332.48 keV. These alloys were chosen because they have good weldability, corrosion resistance, machinability, anodizability and electrical conductivity. Furthermore, these attenuation parameters theoretically computed using PhyX-PSD program developed by Şakar et al. (2020) at the photon energies of 1 keV–1 GeV and compared with the experimental values.

2. MATERIAL AND METHOD

The μ_m, Z_{eff} and N_e values of four different types of aluminum alloys computed using the PhyX-PSD software that utilizes chemical parameters of a mixture materials in the 1 keV−100 GeV energy range. Photon Shielding and Dosimetry (PhyX-PSD) software newly developed by Şakar et al. (2020) is an effective online available software to calculate various shielding and dosimetric parameters such as μm, Z_{eff}, N_e and photon buildup factors for any selected material in the wide energy range of 1 keV–100 GeV (Şakar et al., 2020). The PhyX-PSD is an easy-to-use program that converts all protection and dosimetric parameters above previously introduced in a very understandable and practical method for analysis in MS Excel. The chemical compositions and some physical and mechanical properties of the investigated four types of aluminum alloys are listed in Table 1.

Table 1. The chemical composition and some physical properties for the present aluminum alloys.

Properties	**Aluminum Alloys**			
	5083	5754	6061	6082
Density (g.cm^{-3})	2.65	2.66	2.70	2.71
Thickness (cm)	0.20	0.25	0.30	0.25
Chemical Composition (Wt. %)	Fe; 0,4%	Fe; 0,4%	Fe; 0,5%	Fe; 0,5%
	Si; 0.4%	Si; 0.4%	Si; 0.6-1.0%	Si; 0.7-1.3%
	Cu; 0.1%	Cu; 0.1%	Cu; 0.6-1.1 %	Cu; 0.1%
	Mn; 0.4-1.0%	Mn; 0.5%	Mn; 0.2-0.8%	Mn; 0.4-0.1%
	Mg; 4.0-4.9%	Mg; 2.6-3.6%	Mg; 0.8-1.2%	Mg; 0.6-1.2%
	Zn; 0.25%	Zn; 0.2%	Zn; 0.25%	Zn; 0.2%
	Cr; 0.05-0.25%	Cr; 0.3%	Cr; 0.1%	Cr; 0.15%
	Ti; 0.15%	Ti; 0.15%	Ti; 0.1%	Al; rest
	Al; rest	Al; rest	Al; rest	
Temper	0/H111	0/H111	T6	T6
Yield strength (MPa)	125-145	80-100	240-270	260-310
Tensile strength (MPa)	275-300	190-215	260-310	310-340
Elongation (%50)	22	24	20	19
Hardness (Brinell)	70-75	50-55	95	95

The narrow beam transmission geometry was employed to measure mass attenuation coefficients of 5083, 5754, 6061 and 6082 coded aluminum alloys. The prepared samples were placed between gamma source and the detector with suitable geometrical arrangement as shown in figure 1. The samples were irradiated by 661.66, 1173.23 and 1332.48 keV gamma rays emitted by ^{137}Cs and ^{60}Co radioactive point sources. The incident and transmitted gamma-rays intensities were measured using a NaI(Tl) scintillation detector based on gamma spectrometry system (Cengiz and Caglar, 2016) The data were collected into 1024 channels of a multichannel analyzer and spectra were analyzed with Ortec Maestro software. Measurements were acquired for a period of 1800s with and without the sample and repeated four times for all samples. Figure 2 shows a typical attenuated and unattenuated γ-ray spectrum obtained from ^{137}Cs and ^{60}Co sources for 5083 coded alloy.

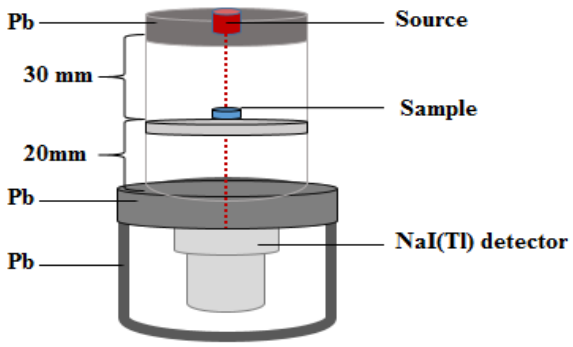

Figure 1. Experimental setup.

The experimental mass attenuation coefficients values of aluminum alloys at different energies were evaluated by the well-known Beer -Lambert law which is given by the equation 1 (Sayyed, 2016; Şakar et al., 2020):

$$I = I_0 e^{-(\frac{\mu}{\rho})x} \quad (1)$$

where, μ/ρ is the mass attenuation coefficient (cm^2g^{-1}), x is the sample thickness (gcm^{-2}), I_0 and I are the unattenuated and attenuated photon intensity, respectively.

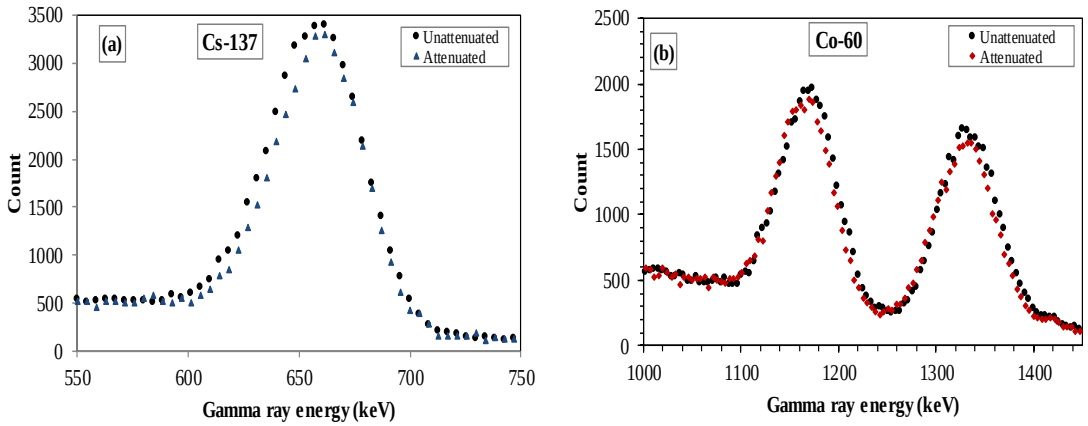

Figure 2. Attenuated and unattenuated gamma ray spectrum obtained from ^{137} Cs and ^{60}Co

2.1. Calculations

The theoretical μ_m values of present alloys were determined using PhyX-PSD program which uses mixture rule given in equation 2;

$$\mu_m = \frac{\mu}{\rho} = \sum_i w_i(\mu_m)_i \quad (2)$$

In this equation, wi and μ_m are the weight fraction and mass attenuation coefficient for individual element in the sample, respectively. The effective atomic number (Z_{eff}) represents the weighted average atomic number of the material consisting of different elements and

directly related to total atomic and electronic cross-sections (σ_a and σ_e) through the following formula (Akkurt, 2009; Elmahroug, Tellili and Souga, 2015; Singh et al., 2018):

$$Z_{eff} = \frac{\sigma_a}{\sigma_e} \tag{3}$$

where

$$\sigma_a = \frac{\sum_i f_i A_i}{N_A} \mu_m \tag{4}$$

and

$$\sigma_e = \frac{1}{N_A} \sum_i \frac{f_i A_i}{Z_i} (\mu_m)_i \tag{5}$$

where N_A is the Avogadro's number, μ_m mass attenuation coefficient of alloy, f_i, A_i, Z_i and $(\mu_m)_i$ denote fractional abundance, atomiweight, mass attenuation coefficient and atomic number of the of the i*th* element in alloy respectively. Also, the effective electron number is closely related to the Z_{eff} and can be computed using the following equation (Akkurt, 2009; Elmahroug et al., 2015; Sayyed et al., 2016):

$$N_e = \frac{\mu_m}{\sigma_e} = \frac{Z_{eff} N_A}{\sum_i \frac{w_i}{A_i}} \tag{6}$$

where N_A is the Avogadro's number, A_i and w_i is atomic weight and fractional weight of the ith element respectively.

3. RESULTS AND DISCUSSION

The μ_m values of 5083, 5754, 6061 and 6082 coded aluminum alloys were measured at 661.66, 1173.23 and 1332.48 keV gamma ray energies and given in Table 2. While the highest experimental μ_m values were seen in 661.66 keV gamma ray energy for all samples, the lowest values were observed in 1332 keV gamma ray energy.

Table 2. The mass attenuation coefficient (μm, cm^2g^{-1}) for the investigated aluminum alloys at 661.66, 1173.23 and 1332.48 keV gamma ray energies.

Aluminum Alloys	Gamma ray Energies (keV)		
	661.66,	**1173.23**	**1332.48**
5083	0.07400	0.05615	0.05419
5754	0.07308	0.05601	0.053903
6061	0.07288	0.05439	0.052966
6082	0.07231	0.05402	0.05242

In Figure 3, it is shown that the experimental μm values of all alloys decrease exponentially with increasing gamma energy.

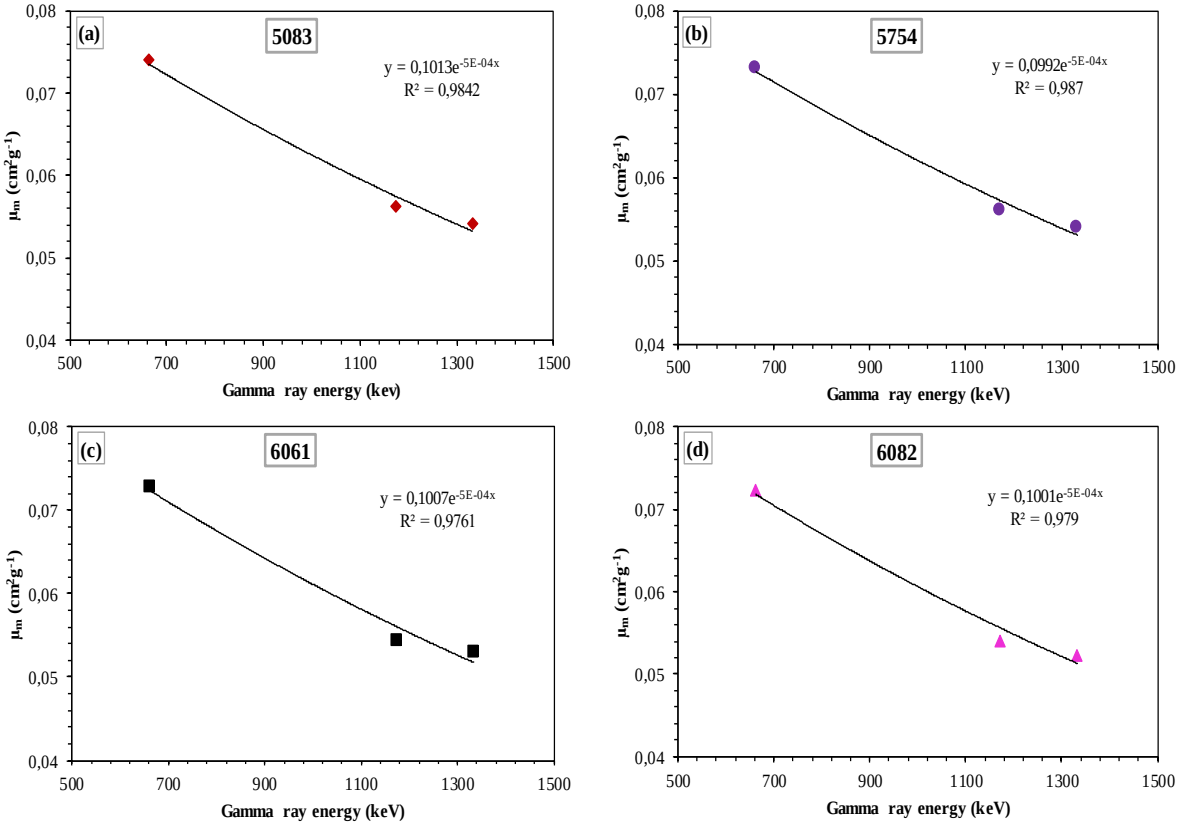

Figure 3. Measured mass attenuation coefficient for (a) 5083 (b) 5754 (c) 6061 (d) 6082 aluminum alloys.

The theoretical μ_m values for studied alloy samples were determined by using PhyX-PSD software in the region of 1keV to 1 GeV. The theoretical results were displayed in figure 4 and compared with the experimental values. As can be seen from the figure 4 (a-d), a good agreement was observed between PhyX-PSD and experimental values (diff. $\leq$ 4.82%). On the other hand, the variations of measured and computed μ_m values with incident photon energy could be seen in figure 4. It observed that the both measured and calculated μ_m values for selected alloys vary with increase in the photon energy.

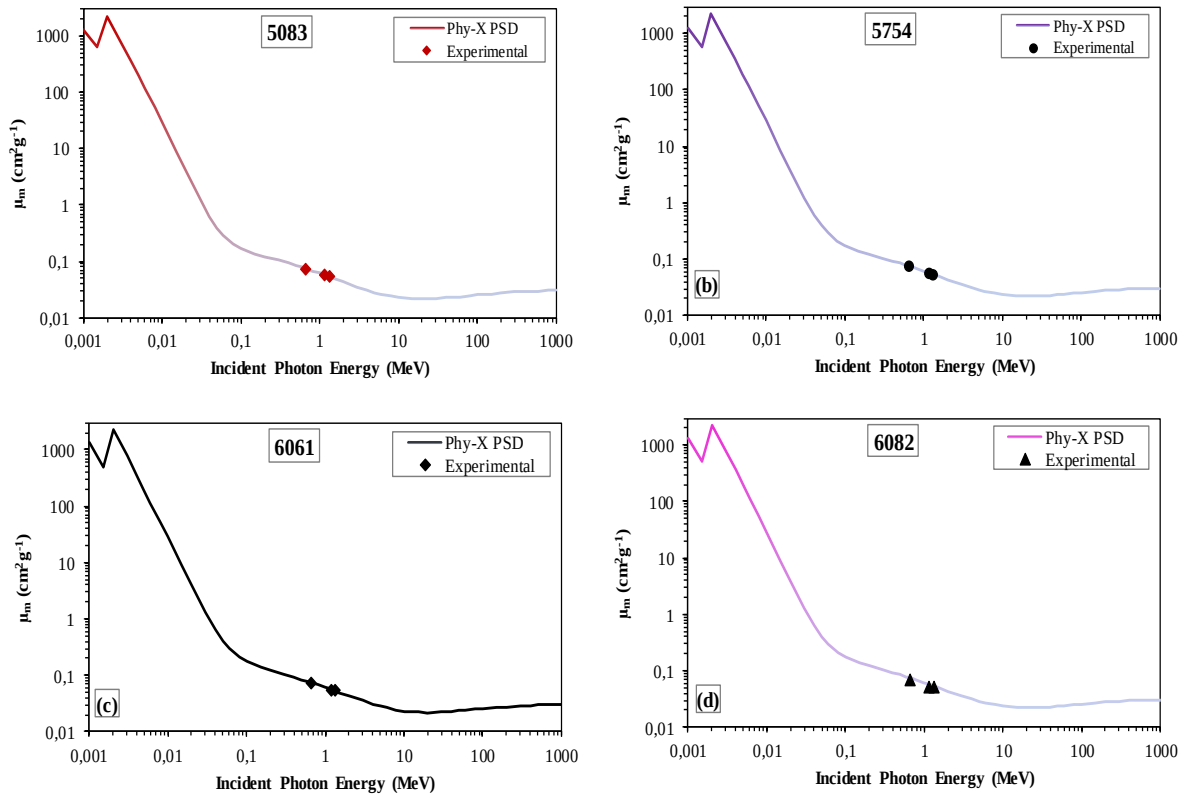

Figure 4. Theoretical and experimental μ_m values of (a) 5083 (b) 5754 (c) 6061 (d) 6082 alloys as a function of photon energies.

According to our findings, it was seen that the μ_m values of four different type aluminum alloys decrease very sharply in the $E \leq 0.1$ MeV region, decrease slowly at 0.1 MeV < E < 6 MeV region and slowly increase at E> 6 MeV region. As early discussed by different researchers, these observed variations may be expressed by the fact that different interaction processes occur between photons and materials in different energies. Because photoelectric absorption is the dominant interaction mechanism in the low energy region (0.1 MeV ≤ photon energy for studied samples) and the photoelectric cross section is inversely proportional to the photon energy ($E^{3.5}$). In the moderate energy region (0.1 MeV < incident photon energy < 6 MeV for investigated samples), Compton scattering gradually becomes the dominant interaction process, and the Compton scattering cross-section changes directly with the atomic number Z of the atom in the absorber material and inversely depend on the photon energy (E^{-1}). In the high energy zone (incident photon energy > 6 MeV for investigated samples), the dominant interaction mechanism between the photon and the material is the pair production and the cross section of this event is proportional to Z^2 (Yorgun and Kavaz, 2018; Sayyed, Kaky, Gaikwad, Agar, Gawai and Baki, 2019b; Şakar et al., 2020). In addition, it observed that the μ_m

values of our investigated alloys are consistent with the consequence acquired by Yıldırım et al. (2016) who are investigated radiation attenuation behavior of some aluminum alloys. For example, they measured μ_m values of 6063 coded aluminum alloys as 0.07351cmg^{-1} for 662 keV gamma ray energy and this value is very close to our values. On the other hand, our results consistent with the result by Narender et al. (2013) who determined experimental mass attenuation coefficient values of 5070 aluminum alloys as 0.0751, 0.05742 and 0.05352 cmg-1 for 661.16, 1173 and 1332 keV energies.

Figure 5 (a-d) show the variation of the Z_{eff} values of the investigated aluminum alloy measured for 661.66, 1173.23 and 1332.48 keV energies and theoretically computed for the energy range of 1kev to 1GeV with photon energy. It can be seen from figure 5 that the experimental Z_{eff} values are compatible with the values obtained from PhyX-PSD. At 661.66, 1173.23 and 1332.48 keV gamma ray energies Z_{eff} values were measured as 12.91, 12.90 and 13.28 for 5083 alloy, 12.77, 12.89 and 13.23 for 5754 alloy 12.86, 12.64 and 13.13 for 6061 alloy and 12.66, 12.45 and 12.88 for 6082 alloy, respectively. PhyX-PSD results of Z_{eff} values at the same gamma ray energies were also determined as 13.046, 13,045 and 13.045 for 5083 alloy 13.66, 13.65 and 13.65 for 5754 alloy 13.11 13.10 and 13.10 for 6061 alloy and 13.13, 13.12 and 13,12 for 6082 alloy, respectively. The maximum difference between theoretical and experimental Z_{eff} values was found to be 4.05 percent for 6082 coded sample at 661.66 keV gamma ray energy. Since the main component of alloys is aluminum, effective atomic number values range from 12 to 14 (ie $12<Z_{eff}<14$). In addition, Z_{eff} values for all alloys are quite high in the low energy region where photoelectric absorption is the strong interaction process and reduces with growing photon energy in moderate energies where Compton scattering is the strong interaction process, and increases gradually at higher energies where pair production is the stronginteraction procedure.

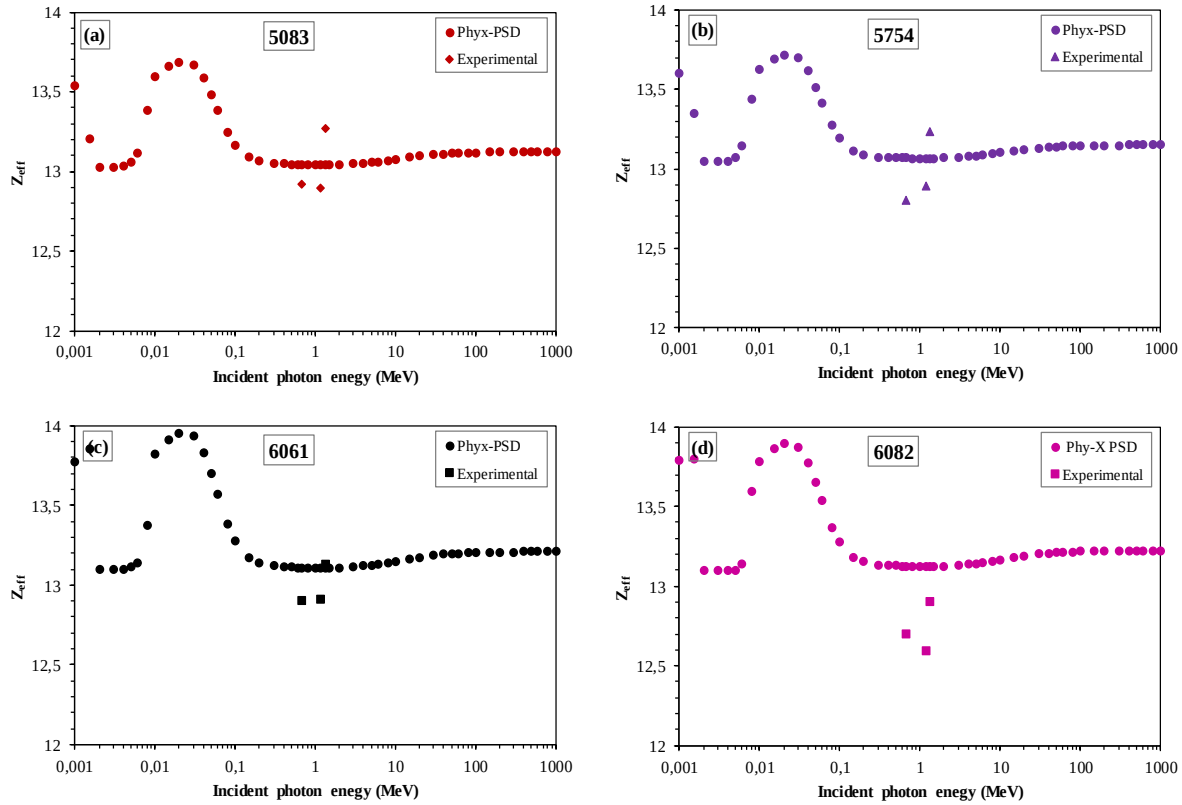

Figure 5. The variations of Z_{eff} for **(a)** 5082, **(b)** 5754, **(c)** 6061 and **(d)** 6082 coded alloys against incident photon energy

The variations of N_e for 5082, 5754, 6061 and 6082 coded samples with energy are given in figure 6 (a-d), respectively. As with the effective atomic number, the theoretical and experimental results of the N_e were found to be compatible with each other. At 661.66, 1173.23 and 1332.48 keV gamma ray energies N_e values were measured as 2.874, 2.870 and 2.55 (×1023electrong-1) for 5083 alloy, 2.838, 2.864 and 2.940 ($\times 10^{23}$electrong^{-1}) for 5754 alloy 2.830, 2.781 and 2.890 ($\times 10^{23}$electrong^{-1}) for 6061 alloy and 2.810, 2.762 and 2.860 ($\times 10^{23}$electrong^{-1}) for 6082 alloy, respectively. PhyX-PSD results of Ne values at the same gamma ray energies were also determined as 2.904, 2.903 and 2.903 ($\times 10^{23}$electrong^{-1}) for 5083 alloy 2.903, 2.902 and 2.902 ($\times 10^{23}$electrong^{-1}) for 5754 alloy 2.901, 2.900 and 2.900 ($\times 10^{23}$electrong^{-1}) for 6061 alloy and 2.902, 2.901 and 2.901 ($\times 10^{23}$electrong^{-1}) for 6082 alloy, respectively. The maximum difference between theoretical and experimental N_e values was found to be 4.15% for 6061 coded sample at 661.66 keV energy. Narender et al. (2013) determined the Z_{eff} values of the 5070 coded aluminum alloy as 13.134 for 661.16, 1173 and 1332 keV gamma ray energies. It was observed that our calculated and measured Z_{eff} values are consistent with the results of Narender et al. (2013). Similarly, the N_e values of the 5070 coded

aluminum alloy for the same energies were reported as 2.904×10^{23} electronsg^{-1} by Narender et al. (2013). It was seen that our obtained theoretical and experimental N_e values were also consistent with their results.

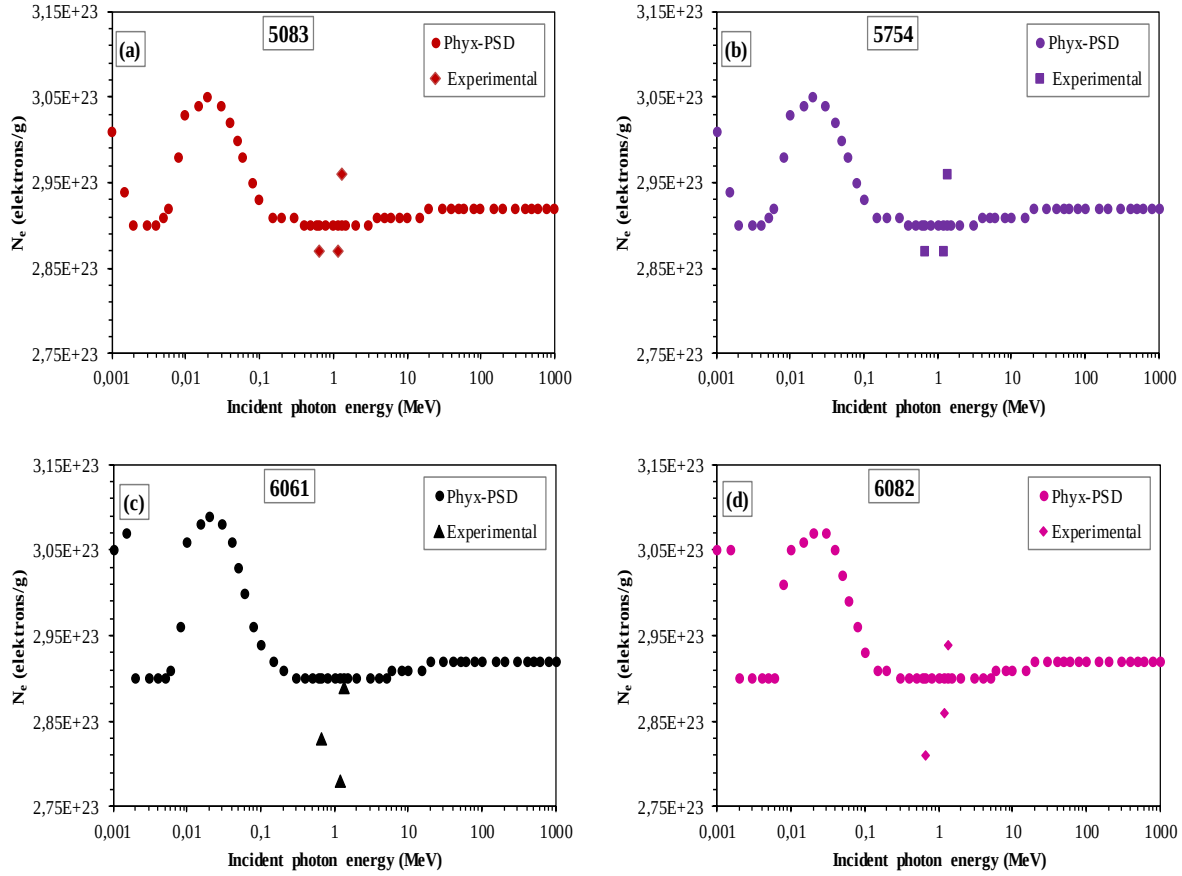

Figure 6. The variations of N_e for **(a)** 5082, **(b)** 5754, **(c)** 6061 and **(d)** 6082 coded alloys with the incident photon energy.

Furthermore, it is seen from figure 6 (a and b) that the Ne values of the aluminum alloys within the scope of the study decrease with increasing photon energy at low energies and the maximum values for N_e are below 100 keV. In the intermediate energies (100 keV – 6 MeV) N_e have minimum values and started to slowly increase above about 6 MeV. This trend observed in N_e values is the same as the trend in Z_{eff} values, because the effective electron density is pretty interrelated to the effective atomic number as it can be seen in equation 5. Hence, obtained trends in this investigation for Z_{eff} and N_e are consistent with the results obtained by Büyükyıldız (2017) who investigated radiological properties of 6061 aluminum alloy and some other shielding materials. On the other hand, it was observed from figures 4, 5 and 6 that there was a sudden jump in the curves of μ_m, Z_{eff} and N_e in the low energy region. This instantaneous splash can be elucidated by the K absorption edge of silicon at 1.839 keV.

4. CONCLUSION

The μ_m, Z_{eff}, and N_e values of 5082, 5754, 6061 and 6082 coded aluminum alloys were experimentally determined at 661.66, 1173.23 and 1332.48 keV gamma ray energies. Also, these parameters theoretically calculated using PhyX-PSD program in the energy region of 1 keV to 1GeV. A decent concurrence was observed among experimental and PhyX-PSD results. It can be concluded that the μ_m, Z_{eff} and N_e values of the aluminum alloys under study depend on the incident photon energy and are influenced by distinct dominant interaction processes (i.e. photoelectric effect, Compton scattering, pair production and etc.) in different energy regions. From the theoretical and experimental results, it was observed that there was no considerable difference among the gamma ray attenuation parameters of the studied aluminum alloys. The results of this work are anticipated to be beneficial in areas like nuclear, aerospace and engineering applications where these aluminum alloys are widely used.

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Effect of ACE Plus Selenium on Total Antioxidant/Oxidant Capacity and Nitric Oxide Levels in Rabbits

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Abstract: Pills including vitamins and minerals have been used as part of a sedentary lifestyle, malnutrition, aging, and age-related illnesses in developed countries. This study specifically focuses on the effect of ACE Plus Selenium given rabbits through intraperitoneal (i.p) injection on their total oxidant, antioxidant capacity (TOC, TAC), and nitric oxide (NO) levels. In this study, 0,5 ml/kg of normal saline was injected into rabbits in the control group and 0,5 ml/kg of ACE Plus Selenium was conducted to the treatment group twice (every other day) via i.p. route. Following the injection, plasmas of blood samples obtained in the second and fourth days were separated, and stored at –20°C until the analysis. Plasma TAC, TOC and NO levels were determined spectrophotometrically. The TOC, TAC and NO levels of the rabbits were compared to the control group statistically. While TOC levels were observed to decrease ($p<0,05$) on the fourth day, the NO levels increased ($p<0,01$) on the second day and became normal on the fourth day. Further no statistical alteration was observed in the TAC levels. As a result, it can be concluded that ACE Plus Selenium had no effect on TAC level, it may decrease TOC levels in parallel to the decline in oxidative stress; further, it can increase NO levels acutely as a result of α-tocopherol and ascorbate ingredients.

Keywords: Multivitamin, Oxidative stress, Antioxidant, Nitric oxide

1. INTRODUCTION

There is increasing evidence that oxidative stress plays a causal role in cancer, cardiovascular and neurological diseases, and aging-related disorders when free radicals are over-produced and/or insufficiently eliminated (Phaniendra et al., 2015; Deveci 2017; Deveci 2018). Free radicals formed as a consequence of the normal metabolism of the cell or by various external factors (food, ionizing radiation, etc.) are neutralized by enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) or by non-enzymatic antioxidants such as A, C, E vitamins, alpha-lipoic acid, ubiquinone and flavonoids (Mercan, 2004; Urso and Clarkson, 2003; Deveci 2019). Oxidants and antioxidants

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are stable in living organisms and any shift in this stability in favor of oxidants causes oxidative stress which is believed to play a causal role in aging and tissue damage associated with various diseases such as cancer, rheumatoid arthritis, Alzheimer's and Parkinson's (Phaniendra et al., 2015; Nur 2017; Deveci 2018). Although NO synthesized from L-arginine by catalysis of nitric oxide synthase (NOS) enzyme is not very reactive, it forms intermediates with damaging effects on biomolecules. Aside from its function as a neurotransmitter and an antioxidant and its role in the regulation of blood pressure, NO also plays a role in ischemia-reperfusion injury, chronic inflammatory bone disease and neurodegenerative diseases when overproduced (Ozcan and Ogun, 2015). The use of antioxidant vitamins such as ascorbic acid, α-tocopherol and β-carotene has become an attractive strategy for reducing the risk of oxidative damage-induced illnesses (Wei et al., 2006) and numerous natural or synthetic antioxidants referred to as exogens are believed to have positive effects on health and disease prevention (Sivoňová et al., 2006).

According to various epidemiological studies, it is suggested that a diet rich in antioxidants may be a strategy for preventing oxidative stress-related diseases (Tabart, 2009). The amount of vitamins and minerals received from foods alters as a result of changes in nutrition patterns of people due to climatic and ecological differences, and cultural and socio-economic factors (Report of a Joint FAO/WHO expert consultation, 1998). Today, especially in the developed countries, people have appealed to supplements that contain both vitamins and minerals owing to their concerns about sedentary lifestyle, malnutrition, aging and aging-related diseases (Fletcher et al., 2002). It is reported that in the United States about seven out of ten Americans spend $ 4 billion every year on products containing 3500 different vitamins and minerals (Erden and Tanyeri, 2004) and that the food supplement market in Europe is expected to grow 9.5% accounting for 7.9 Billion euros by 2020 (Ergen and Bozkurt, 2016).

Given that oxidative stress, which is the balance between pro-oxidative and antioxidative processes, is the cause or consequence of many diseases, oxidant/antioxidant capacity estimation may be important for the health of individuals. Despite the availability in the literature of studies on multivitamins and mineral supplements concerning diseases, there is no study examining the effect of multivitamins and mineral supplements on TAC, TOC and NO levels. This study was conducted to evaluate the effects of ACE Plus Selenium on TOC, TAC and NO levels in rabbits.

2. MATERIAL AND METHOD

The study was confirmed by the Ethics Committee of the Animal Experiments of Kafkas University (Decision no. KAU-CAE / 2012-87). The study was conducted on 18 New Zealand rabbits (*Oryctolagus cuniculus*) with an average live weight of 3.45± 0.4 kg and 14 to 20 months of age. The rabbits were divided into two groups: Control group (n=9) and ACE Plus Selenium Experiment group (n=9). The control group was applied 0.5 ml kg^{-1} normal saline while the treatment group was applied 0.5 ml kg^{-1} ACE Plus Selenium (Provitamin A 20 mg, Vitamin C 200 mg, vitamin E 200 mg, 50 µg selenium) i.p twice every other day. Blood samples were taken from the rabbits on days 2 and 4 following the injection. Their plasma was separated and stored at -20 °C until analysis. TAC and TOC levels in the samples were measured with Rel Assay Diagnostics Assay (Gaziantep-Turkey, Catalog No. RL0017, RL0024) commercial kits developed by Erel (2004; 2005) while NO levels were measured using a spectrophotometer depending on the method suggested by Miranda et al. (2001).

Statistical analysis of the data of the study was evaluated using the SPSS.16 (SPSS 16, USA) package program. Means between the groups were determined by one-way analysis of variance (ANOVA) and differences between the groups were detected by the Tukey test. The results were presented as; mean (±) and standard error (x ± Sx).

3. RESULTS

There was a statistically significant decrease ($p<0.05$) in TOC levels of the experiment group on day 4, and a statistically significant increase in NO levels on day 2, and a decrease back to the normal NO levels on day 4. There was, however, no statistically significant difference in TAC levels between the two groups (Table 1).

Table 1. Plasma TAC, TOC and NO values of ACE Plus Selenium-given rabbits

Parameter	Control	ACE Experimental		P-value
		Day 2	Day 4	
TAC (mmol Trolox Equiv. L^{-1})	0.59 ± 0.04^{a}	0.71 ± 0.06^{a}	0.69 ± 0.05^{a}	ns
TOC (µmol H_2O_2 Equiv.L^{-1})	9.17 ± 1.09^{a}	7.49 ± 0.61^{ab}	6.14 ± 0.58^{b}	*
NO (µmol L^{-1})	17.88 ± 2.28^{b}	27.34 ± 1.73^{a}	21.13 ± 1.75^{b}	**

***:** Differences in the same line are statistically significant ($p<0.05$), **: Differences in the same line are statistically significant ($p<0.01$), ns: Differences in the same line are statistically insignificant

4. DISCUSSION AND CONCLUSION

In vivo, the position of each compound constitutes a substantial factor in preventing the cell from oxidative damage. Being more lipophilic, β- carotene is located in the interior of the membranes and lipoproteins; α- tocopherol is located in the vicinity of the membrane and water interface despite its lipophilic nature while ascorbic acid is located in the extracellular matrix and hydrophilic parts of the cells. Thus, ascorbic acid constitutes the first line of defense (Zhang and Omaye, 2001).

Antioxidant vitamins are thought to be more effective when used together than when used individually. For example, a study using a membrane model suggested that the combination of β-carotene and α-tocopherol inhibited radical-induced lipid peroxidation and that this inhibition was more pronounced when the two compounds were used together than when used individually (Palozza and Krinsky, 1992) while another study suggested that ascorbic acid when added to the test environment, protected low-density lipoprotein (LDL) from oxidation synergistically together with β-carotene (Packer, 1993).

There is no precise information regarding the contributions of multivitamin and mineral supplements to prevent diseases and protect health. In some studies, β-carotene has been reported to significantly increase the incidence of lung cancer (ATBC Study Group, 1994), and it has been claimed that E, C and partially high-dose supplementation of vitamin A increases the risk of mortality and therefore does not have any benefit (Bjelakovic et al., 2015). Yet, there are some objections to these arguments that they cannot lead to the same effects in healthy individuals due to disease conditions (inflammation, etc.). For example, it has been suggested that iron is released from ferrite during inflammation and sepsis (Biemond et al., 1984) while vitamin C reacts easily with Fe^{+3} and forms ascorbyl radical and Fe^{+2} which in turn reacts with H_2O_2 and generates the $HO^{\cdot}$ radical which is extremely harmful to biomolecules (Childs et al., 2001).

Studies in volunteers suggested that multivitamins and mineral supplements decreased aging-related oxidative DNA damage (Ribeiro et al., 2007) and when used together with fish oil, reduced F_2- isoprostane level as a parameter of oxidative stress (Pipingas et al., 2015). A recent study reported that the combined use of vitamin E and selenium nanoparticles increased cock sperm quality and significantly reduced the amount of lipid peroxidation after freeze-thawing (Safa et al., 2016). Henning et al. (2000) observed that 3 weeks of multivitamin and mineral supplement use did not change the plasma antioxidant capacity in young volunteers.

This study shows that ACE plus Selenium does not affect TAC levels while leads to a statistically significant decrease in TOC levels on day 4. It has been concluded that the reduction

in TOC levels may be because of the synergistic effect of antioxidants in the content of multivitamins and mineral supplements used in the study (Palozza and Krinsky, 1992; Packer, 1993).

It is stated that NO formation also depends on tetrahydrobiopterin (BH_4) which is one of the intracellular cofactors and a reduction in BH_4 leads to short-term endothelial dysfunctions, however, BH_4 support can increase cellular NO formation and restore endothelium-dependent relaxation (Ignarro, 2010). It is showed that BH_4 deficiency may be related to oxidative stress in the vascular system; and ascorbic acid may protect BH_4 from oxidative stress (dUscio et al., 2003), and increase NO synthesis by regressing the neutral trihydrobiopterin generated by the radical reactions back to BH_4 (Patel et al., 2002).

Other studies showing the interaction between NO and antioxidants report that NO protected α-tocopherol from oxidation and inhibited the lipid peroxidation process in liposomes together with α-tocopherol (Rubbo et al., 2000); and recovered UV-A induced cell damage in fibroblast cells together with ascorbic acid (Oplander et al., 2007).

The statistically significant increase in the amount of NO on day 2 and then the decrease back to the normal level observed in this study may be due to α-tocopherol and ascorbic acid (Patel et al., 2002; dUscio et al., 2003; Heller et al., 2004).

It has been concluded that ACE Plus Selenium does not have any effect on TAC levels, and may result in a decrease in TOC levels due to reduced oxidative stress and may acutely increase NO levels owing to its α-tocopherol and ascorbate content.

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Determination of Cytotoxicity of Zinc 2-Bromobenzoate with Nicotinamide and N,N'-Diethylnicotinamide Complexes

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Abstract: Benzoic acid and its derivatives and their metal complexes, which have antimicrobial, anticancer, antituberculosis and antioxidant properties, are biologically active molecules. Although there are many studies on the biological activity of these compounds, studies on the determination of their toxicity are limited. In the presented study, the cytotoxic properties of the previously synthesized diaquabis(2-bromobenzoato-κO)bis(nicotinamide-κN1)zinc(II) (ZnBrBANA) and diaquabis(2-bromobenzoato-κO)bis(N,N'-diethylnicotinamide-κN1)zinc(II) (ZnBrBADENA) complexes were investigated. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, a colorimetric method, was used to determine the cytotoxicity of complexes on human peripheral lymphocyte cells. Besides, cytotoxicity of dimethylsulfoxide (DMSO) which is crystal solvent and 2-bromobenzoic acid (BrBA), nicotinamide (NA), and N,N'-dietyhlnicotinamide (DENA) which are starting compounds of the complexes was also evaluated. According to the results of MTT method, It has been determined that both complexes and starting components except BrBA cause cytotoxicity on lymphocyte cells at the concentration range of 62.5-500 ppm. In addition, it was determined that the BrBA and DMSO at the same concentration range do not show any cytotoxic effect on lymphocyte cells. It was observed that the synthesized complexes were more toxic at each concentration than the starting components. Therefore, the toxic effects of the complexes used as drug active ingredients should be followed up with new studies.

Keywords: Cytotoxicity, MTT assay, 2-Bromobenzoic acid, Zinc Complex

1. INTRODUCTION

In recent years, the cytotoxic and genotoxic properties of metal complexes and their roles in biological processes have been investigated (Mjos and Orvig, 2014). One of the most important reasons for this is the ability of metal drugs to target to DNA. The biological activity of these compounds depends on their structure and the ligands included. But crystal engineering must take into account any interaction, both weak and strong, to clarify molecular architecture and crystal packaging (Lu et al., 2019). Investigation of the relationship between the structure of compounds and their biological activities makes it possible to synthesize new drugs suitable for the purpose (Balan et al., 2020). Zinc complexes attract attention with their crystal structures as well as their biological and physical properties. In recent years, many zinc complexes containing biologically active ligands have been synthesized by different research groups and

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their crystal structures have been investigated (Hökelek et al., 2009; Özbek et al., 2019; Pucci et al., 2013; Sertçelik et al. 2012; 2018; Taşdemir et al., 2016). Zinc (II) complexes are used as DNA binders, tumor photosensitizers, antidiabetic, antifungal, antioxidant and antibacterial agents (Liguori et al., 2010; Pucci et al., 2013). Moreover, in recent studies, Zn (II) complexes have reported that they can be anticancer agents due to their low toxicity and less side effects (Liguori et al., 2010; Pucci et al., 2013).

Metal complexes of benzoic acid and its derivatives have great abilities in various fields (Bakhtiar and Ochiai, 1999; Heine and Müller-Buschbaum, 2013; Krishna, 2015; Li et al., 2013; Rimoldi et al., 2017; Zhu et al., 2017). For the rational design, construction of their supramolecular architecture and properties, auxiliary ligands used as well as the main ligands are also important (Jozef et al., 2016). Used as auxiliary ligands, N-donor heterocyclic compounds are a component of various vitamins and drugs and play an important role in many biological systems. Nicotinamide, the body form of vitamin B3, is a N-donor ligand used in the treatment of some skin diseases. Another N-donor ligand with a similar structure is N,N'-diethylnicotinamide, which is used as a respiratory stimulator in medicine (Cavicchi, 1959; Krajníková et al., 2011). While the crystal structure of metal arylcarboxylates with different co-ligands changes, it is found that the biological activities and toxicity profiles of the complexes change with the change of these ligands (Wang et al., 2020). Drug active ingredients are carried by the blood tissue in the body. Therefore, these active substances first come into contact with blood tissue cells. Therefore, the cytotoxic effects of the two complexes used in this study on lymphocyte cells were investigated. The aim of this study, to determine the cytotoxicity effects of previously synthesized zinc 2-bromobenzoate complexes with nicotinamide and N,N'-diethylnicotinamide were used by MTT test method on lymphocyte cells.

2. MATERIAL AND METHOD

2.1. Materials

The two complexes used in the study were a previously synthesized and their structures were determined (Hökelek et al. 2009a; 2009b). The structures of the complexes were given in Figure 1 and 2. Phosphate Buffered Saline, Antibiotic Antimycotic Solution, L-Glutamine solution, Histopaque-1077 and Dimethylsulfoxide (Sigma-Aldrich), BIOAMF-1 medium and BIOAMF-1 supplement (Biological Industries) and MTT Cell Proliferation Assay Kit (Cayman Chemical) were purchased commercially. Solutions of the complexes at concentrations of 62.5, 125, 250 and 500 ppm were prepared in DMSO. Nüve Steamart OT 40L autoclave, Nüve BM

101 Water bath, ISOLAB vortex mixer, HETTICH EBA 200 centrifuge device, Panasonic MCO-170AICUVH-PE CO_2 Incubator and BioTek Epoch Spectrophotometer were used.

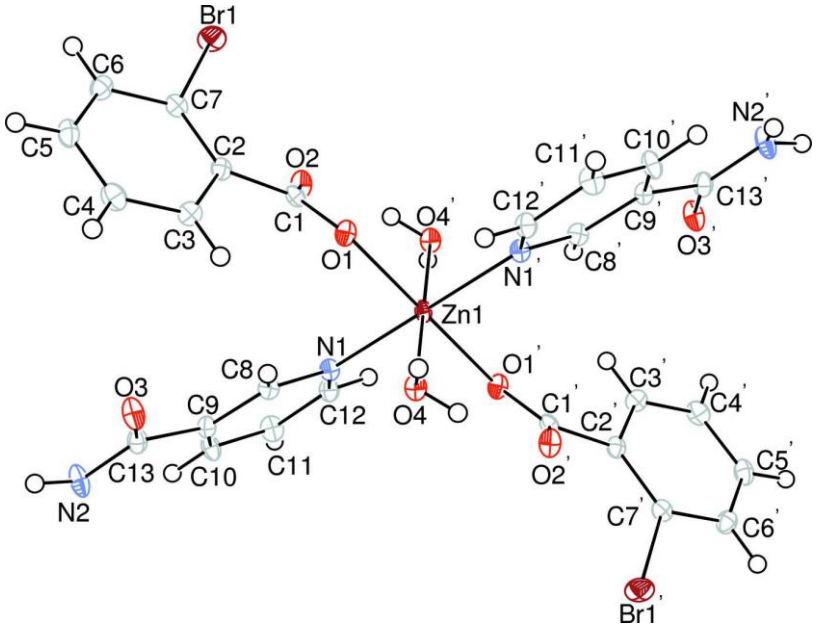

Figure 1. The structure of diaquabis(2-bromobenzoato-κO)bis(nicotinamide-κN^1)zinc(II)(Hökelek et al., 2009a)

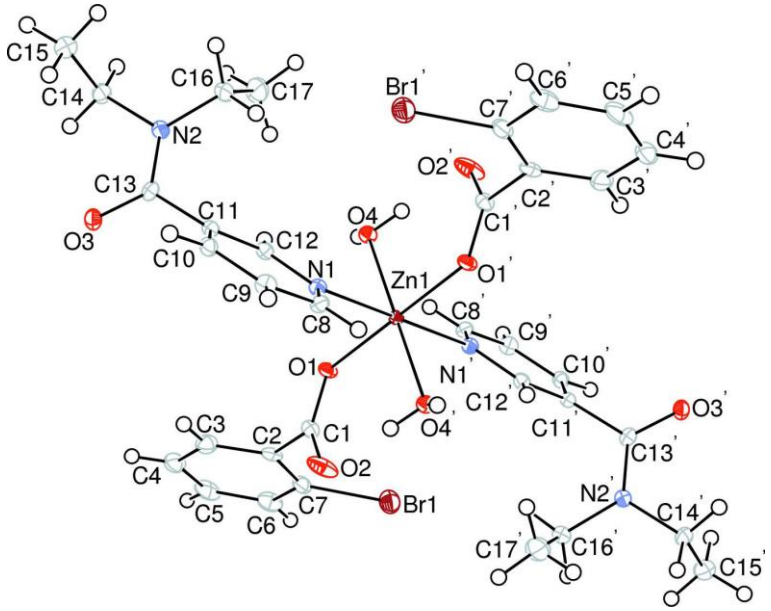

Figure 2. The structure of diaquabis(2-bromobenzoato-κO)bis(N,N'-diethylnicotinamide-κN^1)zinc(II)(Hökelek et al., 2009b)

2.2. Method

Lymphocyte cells to be used in experiments were isolated from a human whole blood sample (Öztürk, 2019) and cell count was done using Thoma slide. The culture medium was

prepared from the a mixture of amnion cell culture medium (75 mL), supplement (15 mL), penicillin + streptomycin + amphotericin B (Antibiotic Antimycotic Solution) (1.5 mL) and L-glutamine (2 mL) into a sterile tube. After that culture medium (100 µL) and cell suspension (100 µL) (50000 cells/well) were added to the 96-well plates, respectively. The cells were incubated to proliferate and adhere to the surface for 24 hours in 5 % CO_2 incubator at 37 °C. The solutions of the complexes at 500, 250, 125 and 62.5 ppm concentrations were prepared in DMSO. After 24 h incubation, 100 µL aliquots of different concentrations of the complexes were added to the wells. The cell cultures were incubated at 37 °C for a day in the incubator. 24 hours later 10 µL of MTT reagent was added to each well and the plates were gently mixed on the shaker. Incubation was continued for 3 hours. Formed formazan crystals were seen in the bottom of the wells. Then, the medium in the well was completely removed and 200 µL DMSO was added to each well to dissolve the formazan crystals. It was kept in the incubator at 37 °C for 24 hours. At the end of the incubation, the absorbance values were recorded by UV-Vis spectrophotometer at 570 nm.

2.3. Statistical analysis

The data obtained from tests were analyzed with IBM SPSS statistics for Windows package program (v.18.0, IBM Corp., Armonk, New York, USA). Two-way ANOVA (Tukey) was used to evaluate whether any treatment significantly differed from the control or each other's. Statistically significance level was accepted at % 95 ($p<0.05$).

3. RESULTS

3.1. MTT Assay

MTT test is one of the most important pre-screening methods to investigate cell proliferation and anticancer activity of natural products and synthetic materials in the search for new drugs (Jamalian et al., 2011; Mosmann, 1983; Teixeira et al., 2007). In this context, MTT test's results of the complexes, solvent and starting components were assessed. The values of absorbance were recorded at 570 nm by spectrophotometer. The change in cell viability was compared with the cell control group and percentage of inhibition values were calculated according to the following equation (1). The values were given in Table 1 and Figure 3.

$$Inhibition\ percentage\ (\%) = \frac{CV}{CVCC} x100 \quad (\mathbf{1})$$

(CV= Cell viability at the test concentrations and CVCC = Cell viability in cell control)

Table 1. Percentage of Cell viability (CV) and Cell death (CD) values at the test concentrations

		500 ppm	250 ppm	125 ppm	62,5 ppm
Cell Control	**CV**	100.00	100.00	100.00	100.00
	CD	–	–	–	–
DMSO	**CV**	102.24	102.24	102.24	102.24
	CD	–	–	–	–
ZnBrBANA	**CV**	56.50	63.21	63.19	63.30
	CD	43.50	36.79	36.81	36.70
ZnBrBADENA	**CV**	66.03	76.23	73.32	86.01
	CD	33.97	23.77	26.68	13.99
NA	**CV**	77.39	77.86	78.75	86.17
	CD	22.61	22.14	21.25	13.83
DENA	**CV**	80.68	84.57	83.93	97.88
	CD	19.32	15.43	16.07	2.12
BrBA	**CV**	118.75	104.80	104.42	105.00
	CD	–	–	–	–

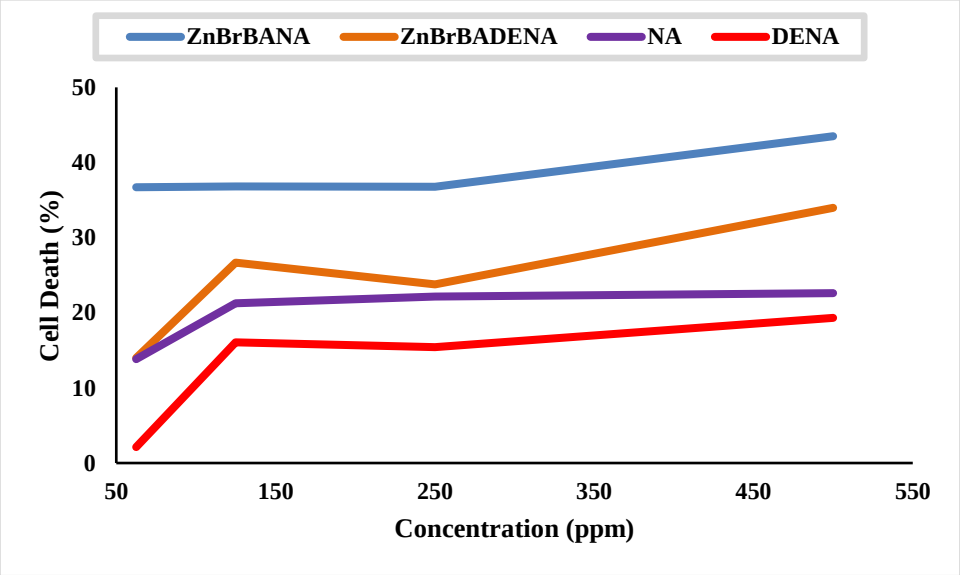

Figure 3. Change in the cell death according to the increasing concentrations

It was determined that DMSO used as solvent did not cause any cell death. Similarly, it was also found that 2-bromobenzoic acid, which is the primary ligand, did not have a cytotoxic effect on lymphocyte cells. NA, the co-ligand in crystal structure of ZnBrBANA, was cytotoxic at all concentrations. While the other co-ligand DENA did not cause cytotoxicity at 62.5 ppm, an average of 16.94 % cell death occurred in the 125-500 ppm concentration range. Cytotoxicity of ZnBrBANA and ZnBrBADENA complexes on lymphocyte cells was compared to each other and to the starting compounds (Figure 4-6). According to the results obtained, both complexes

caused cytotoxicity on these cells at the concentration range of 62.5-500 ppm. According to the results obtained, both complexes caused cytotoxicity on these cells at the concentration range of 62.5-500 ppm. ZnBrBANA complex is more toxic at 500 ppm than other concentrations. There is no statistically significant difference at concentrations of 250, 125 and 62.5 ppm.

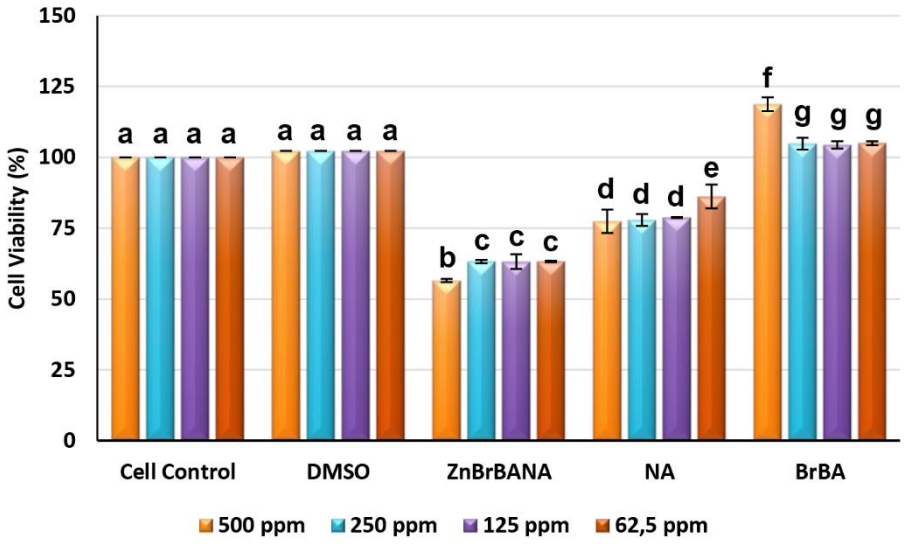

Figure 4. Comparison of cytotoxicity of ZnBrBANA complex with the starting compounds (Different letters on the columns were significantly different at $p<0.05$)

Similarly, the ZNBrBADENA complex showed the highest toxicity at 500 ppm concentration. There was no statistically significant difference between the cell death ratios at 125 ppm and 250 ppm of this complex. ZnBrBADENA at 62.5 ppm was determined to cause less toxicity. The cytotoxicity of both complexes increases with the increasing concentration. The toxicity of the ZnBrBANA and ZnBrBADENA complexes compared to each other, the complex containing the DENA ligand was found to be less toxic. It is clear that this is related to the toxicity of the co-ligands contained in the complexes. In some studies, it has been reported that the toxicity of compounds that cause approximately 10 % cell death in normal cells can be neglected and these compounds can be used as anticarcinogenic agents (Bhattacharyya et al., 2019; Nashre-ul-Islam et al., 2019). Since the ZnBrBADENA complex causes 13 % cell death at 62.5 ppm, it is thought that it could be used as an anticarcinogenic agent in future studies. There are few studies in the literature on the determination of cytotoxicity of zinc arylcarboxylates with N-donor ligands on lymphocyte cells.

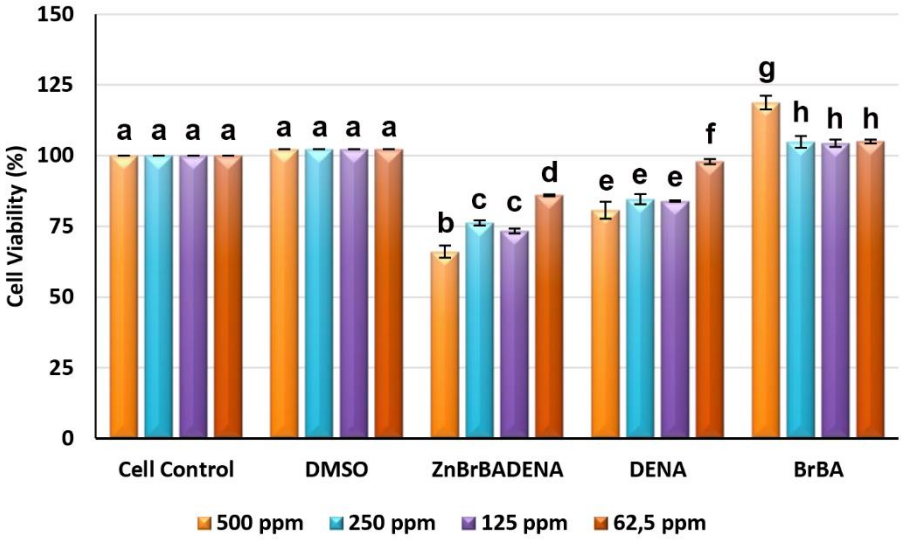

Figure 5. Comparison of cytotoxicity of ZnBrBADENA complex with the starting compounds (Different letters on the columns were significantly different at $p<0.05$).

There is only one study in the literature on the determination of the cytotoxicity of zinc arylcarboxylates with pyridine derivative complexes on normal lymphocyte cells. It was reported that the zinc 2-fluorobenzoic acid nicotinamide complex decreased cell viability with increasing concentration at the 1.17-18.67 mM concentration range (Ozturk and Akbaba, 2019). In addition, it was determined that zinc 2-fluorobenzoate nicotinamide complex caused 13.80-93.58 % cell death in the concentration range of 1250-20000 ppm. Cell death caused by this complex at 10000 ppm is less than the toxicity caused by ZNBrBANA and ZnBrBADENA complexes at 500 ppm (Ozturk and Akbaba, 2019). ZnBrBANA and ZnBrBADENA complexes used in this study caused 43.5 % and 33.97 % cell death at 500 ppm, respectively. Generally, although metal complexes are recommended as an anticarcinogenic drug, their cytotoxic effects on normal cells are not investigated. The two complexes used in this study are not recommended to be used as an anticarcinogenic drug because they cause toxicity on lymphocyte cells at high concentrations (500 ppm).

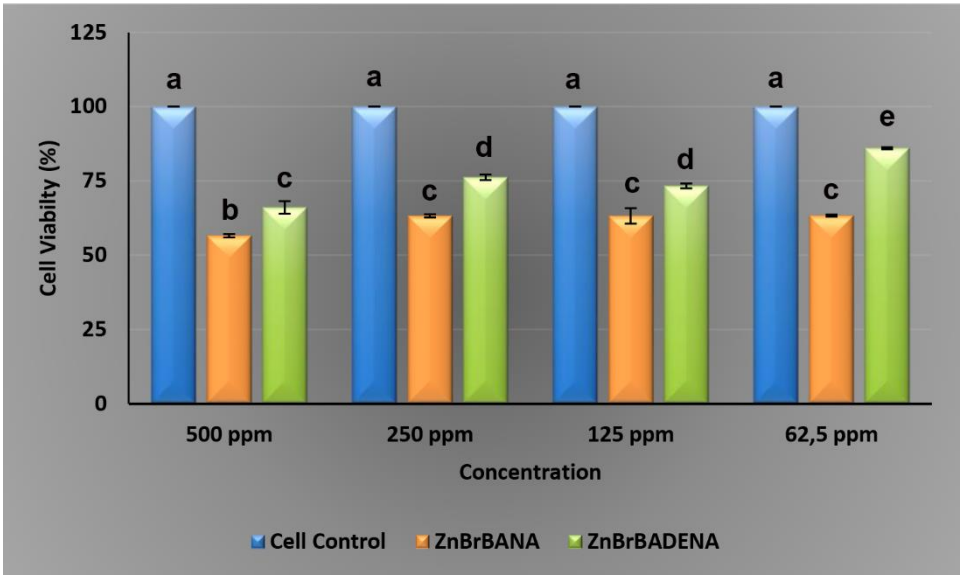

Figure 6. Comparison of cytotoxicity of ZnBrBADENA and ZnBrBANA complexes with cell control (Different letters on the columns were significantly different at $p<0.05$).

4. CONCLUSION

It has been supported by many studies that metal arylcarboxylates are biologically active compounds in the last three decades. Although these compounds are recommended for use as new drug active ingredients, they must pass many tests for their active use. In this context, it is essential to know the cytogenotoxicity of the compounds. In many studies, a limited number of studies have been carried out on the toxicity of these compounds, which are stated to be effective against cancer cell lines, on normal cells. In this context, in this study, the cytotoxicity of Zinc (II) 2-bromobenzoate nicotinamide/N,N'-diethylnicotinamide complexes on lymphocyte cells in the concentration range of 62.5-500 ppm was investigated by MTT test. The ZnBrBANA complex was found to be toxic at all concentrations and the ZnBrBADENA complex caused moderate cell death at other concentrations except 62.5 ppm. The cytotoxicity of the complexes increases with increasing concentration. At 62.5 ppm, the ZnBrBADENA complex is negligible toxic. We propose further studies to evaluate this compound, which causes 13.99 % cell death at this concentration, as DNA binders, tumor photosensitizers, antidiabetic, antifungal, antioxidant and antibacterial agent.

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Topraktan Kars Halkının Tükettiği Buğday Ununa Doğal Radyonüklidlerin Transfer Faktörlerinin Belirlenmesi

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Öz: Bu çalışmada ^{226}Ra, ^{232}Th ve ^{40}K aktivite konsantrasyonları, Kars'tan alınan toplam 45 tane toprak numunesinde ve bu incelenen topraklarda yetiştirilen buğdaylardan üretilen buğday unu örneklerinde ölçülmüştür. Gama spektrometrik analizler sonucunda, toprak örneklerindeki ^{226}Ra, ^{232}Th ve ^{40}K doğal radyonüklidlerinin ortalama aktivite konsantrasyonları sırasıyla, 20,1±8,2; 31,2±7,1 ve 496,1±35,6 Bqkg^{-1} olduğu bulundu. Yöre halkı tarafından ağırlıklı olarak ekmek yapımında kullanılan buğday unu örneklerindeki ortalama aktivite konsantrasyonları ise sırasıyla, 11,0±2,2; 10,8±2,3 ve 304,1±25,5 Bqkg^{-1} olarak bulundu. Topraktan-bitkiye transfer faktörleri, toprakta ve gıdalarda radyoaktivitenin varlığından dolayı çevresel etkinin değerlendirilmesinde temel öneme sahiptir. Bu nedenle, buğday unu örnekleri için bu doğal radyonüklidlerin topraktan-bitkiye transfer faktörleri değerlendirildi. Topraktan-buğday ununa ^{226}Ra, ^{232}Th ve ^{40}K radyonüklidlerinin transfer faktörleri sırasıyla, 0,30±0,10 ile 1,29±0,95; 0,15 ± 0,07 ile 0,86±0,28 ve 0,45 ± 0,07 ile 0,83 ±0,15 aralıklarında olduğu tespit edildi. Çalışma alanındaki doğal radyonüklidlerin aktivite konsantrasyonlarının ülkemizde ve dünyanın farklı bölgelerinde yapılan benzer çalışmaların sonuçları ile uyumlu olduğu görülmüştür.

Anahtar Kelimeler: Toprak, Buğday unu, Doğal radyoaktivite, NaI(Tl) dedektör, Transfer Faktörü

Determination of Transfer Factors of Natural Radionuclides from Soil-to-Wheat Flour Consumed by Kars People

Abstract: In this study, ^{226}Ra, ^{232}Th and ^{40}K activity concentrations were measured in a total of 45 soil samples taken from Kars and wheat flour samples produced from wheat grown in these soils. As a result of gamma spectrometric analysis, the mean activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K natural radionuclides in soil samples were found to be 20.1 ± 8.2, 31.2 ± 7.1, 496.1 ± 35.6 Bqkg^{-1}, respectively. The average activity concentrations in wheat flour samples used mainly in bread making by the local people were found to be 11.0 ± 2.2, 10.8 ± 2.3 and 304.1 ± 25.5 Bqkg^{-1}, respectively. Soil-to-plant transfer factors are of fundamental importance in the assessment of environmental impact due to the presence of radioactivity in soil and food. Therefore, soil-to-plant transfer factors of these natural radionuclides were evaluated for wheat flour samples. The transfer factors of ^{226}Ra, ^{232}Th and ^{40}K radionuclides from soil to wheat flour were determined to be in the range of 0.30 ± 0.10 to 1.29 ± 0.95, 0.15 ± 0.07

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to 0.86 ± 0.28 and 0.45 ± 0.07 to 0.83 ± 0.15, respectively. It has been observed that the activity concentrations of natural radionuclides in the study area are consistent with the results of similar studies conducted in our country and in different parts of the world.

Keywords: Soil, Wheat flour, Natural Radioactivity, NaI(Tl) Detector, Transfer Factor

1. GİRİŞ

Çevredeki doğal ve yapay radyonüklid dağılımının ve bu radyonüklidlerin oluşturduğu radyoaktivite seviyelerinin belirlenmesi, insanların maruz kaldığı hem karasal hem de yapay kaynaklı iyonlaştırıcı radyasyonun etkilerinin belirlenebilmesi için büyük önem taşımaktadır(Agbalagba ve ark., 2012; Sroor ve ark., 2001). Bununla birlikte, topraktaki radyoaktivite seviyelerinin belirlenmesi herhangi bir radyoaktif serpinti ile eşzamanlı olarak doğal background aktivitesindeki değişiklikleri tespit edebilmesini ve çevrede olası herhangi bir radyoaktif serpintinin izlenmesini sağlayacaktır.

Toprak radyoaktivitesi temel olarak gama ışını yayımlayan ^{40}K, ^{226}Ra ve ^{232}Th doğal radyonüklidlerden ve yapay bir radyonüklid olan ^{137}Cs'den kaynaklanmaktadır. Toprak örneklerinde bulunan bu radyonüklidler, besin zinciri yoluyla insan vücudunda iç ışınlamaya neden olmaktadır çünkü çoğu radyonüklid insan vücuduna toprak-bitki-insan zinciriyle taşınmaktadır (Krmar ve ark., 2013). Bu nedenle, toprak radyoaktivitesi ve doğal radyonüklidlerin topraktan-bitkiye transfer faktörleri (TF), insan popülasyonunun doğal ve yapay kaynaklı gama ışını maruziyetinin kapsamlı bir şekilde değerlendirilmesinin yanı sıra radyonüklidlerin hareketlilik, transferler ve yer değiştirme gibi uzun vadeli davranışlarının incelenmesinde önemli rol oynamaktadır.

Son yıllarda, toprak ve bitki örneklerindeki ^{226}Ra, ^{232}Th, ^{40}K ve ^{137}Cs aktivite konsantrasyonları ve bunların topraktan-bitkiye transfer faktörleri (TF) birçok araştırmacı tarafından incelenmiştir. Örneğin, Suudi Arabistan'ın üç farklı bölgesinden (Buraidah, Al-Zulfi ve Al-Majmaah bölgelerinden) toplanan toprak örneklerinin ve bu bölgelerdeki hurma çiftliklerinden toplanan 9 hurma örneğinin doğal radyoaktivite seviyeleri incelenmiştir. Yapılan analizler sonucunda toprak örneklerinden ^{226}Ra, ^{232}Th, ^{40}K ortalama aktivite konsantrasyonları sırasıyla, 12.8 ± 2.2, 102 ± 2.1, 329 ±87 ve 0.28±0.10 Bqkg^{-1} olarak ölçülmüş ve hurma örneklerinde ^{137}Cs radyoaktif çekirdeğine rastlanmamıştır. Ayrıca çalışmada ^{276}Ra, ^{232}Th ve ^{40}K radyoaktif çekirdeklerinin transfer faktörlerinin geometrik ortalamaları sırasıyla 0.33, 0.21 ve 0.51 olarak bulunmuştur (Abu Shayeb ve ark., 2018). Pakistan'da yapılan çalışmada toprak örneklerinin ve buğday, patates gibi yerel halkın günlük beslenmelerinde kullandıkları gıda ürünlerinin ^{226}Ra, ^{232}Th, ^{40}K ve ^{137}Cs aktiviteleri ölçülmüştür. Toprak örneklerindeki,^{226}Ra aktivite konsantrasyonu 30.0 Bqkg^{-1} ile 81.2 Bqkg^{-1} değerleri arasında ortalama 56.2 Bqkg^{-1},

^{232}Th aktivite konsantrasyonu 31.4 Bqkg^{-1} ile 78.5 Bqkg^{-1} değerleri arasında ortalama 58.5 Bqkg^{-1}, ^{40}K aktivite konsantrasyonu 308.8 Bqkg^{-1} ile 2177.6 Bqkg^{-1} değerleri arasında ortalama 851.9 Bqkg^{-1} ve ^{137}Cs aktivite konsantrasyonu ise 1.3 Bqkg^{-1} ile 46.8 Bqkg^{-1} değerleri arasında ortalama 13.39 Bqkg^{-1} olarak ölçülmüştür. Çalışmada incelenen toprak örneklerindeki ^{226}Ra, ^{232}Th ve ^{40}K ortalama aktivite konsantrasyonlarının dünya ortalama değerlerinin üzerinde olduğu görülmüştür. Sonuçlar bu radyoaktif çekirdeklerin konsantrasyonlarının bölgede yaşayan insanların sağlığı açısından bir risk oluşturmadığını açıkça göstermiştir. Ayrıca çalışmada bu radyoaktif çekirdeklerin topraktan gıda ürünlerine transfer faktörleri de çalışılmıştır. ^{40}K, ^{226}Ra, ^{232}Th ve ^{137}Cs radyoaktif çekirdeklerinin transfer faktörleri sırasıyla 0.17, 0.07, 0.16 ve 0.23 olarak bulunmuştur. Bu çalışmada iç ve dış tehlike indeksleri hesaplanmış ve ortalama değerleri sırasıyla 0.70 ve 0.55 olarak bulunmuştur. Yapılan doz hesaplamaları sonucunda çalışma bölgesindeki doğal radyoaktif çekirdeklerin aktivite konsantrasyonlarının nominal değerlerde olduğu ve bölge halkı için sağlık riski oluşturmayacağı bildirilmiştir (Khan Hasan ve ark., 2010). Kars ilinin Digor ilçesinde toplanan toprak ve mera bitkisi örneklerinin doğal radyoaktivite seviyeleri ölçülerek doğal radyonüklidlerin topraktan bitkiye transfer faktörleri hesaplanmıştır. İncelenen toprak örneklerinde ^{266}Ra aktivite konsantrasyonu 60.2 ± 12.5 Bqkg^{-1} ile 98.1 ± 13.3 Bqkg^{-1} değerleri arasında ortalama 80.1 ± 13.8 Bqkg^{-1}, ^{232}Th aktivite konsantrasyonu 54.7 ± 11.6 Bqkg^{-1} ile 81.1 ± 13.3 Bqkg^{-1} değerleri arasında ortalama 65.7 ± 12.6 Bqkg^{-1} ve ^{40}K aktivite konsantrasyonu 450 ± 38.5 Bqkg^{-1} ile 736.6 ± 50.2 Bqkg^{-1} değerleri arasında ortalama 617.0 ± 45.5 Bqkg^{-1} olarak bulunmuştur. Mera otu örneklerindeki ^{226}Ra,^{233}Th, ve ^{40}K aktivite konsantrasyonları sırasıyla 21.8 ± 6.5- 49.6± 13.4 Bqkg^{-1} 51.9 ± 113.5 – 127.7 ± 23.8 Bqkg^{-1} ve 309.5 ± 33.5-807.3 ± 64.4 Bqkg^{-1} aralıklarında bulunmuştur. Ayrıca çalışmada topraktan mera otuna transfer faktörleri hesaplanmış ve transfer faktörleri ^{226}Ra için 0.26 ile 0.69 aralığında, ^{232}Th için 0.64 ile 1.99 aralığında ve ^{40}K için 0.64 ile 1.40 aralığında bulunmuştur. Çalışma sonucunda ^{226}Ra bitki gövde ve yapraklarına oranla köklerde tutulduğu, ^{232}Th aktivite konsantrasyonunun sap>yaprak>kök olarak azalma eğiliminde olduğu ve ^{40}K aktivite konsantrasyonunun bitki saplarında daha yüksek olduğu bildirilmiştir (Bilgici Cengiz, 2018). Gediz nehri havzasında tarım toprağı örneklerinin yanı sıra biber, patlıcan, domates gibi sebze örneklerinin ^{226}Ra, ^{232}Th ve ^{40}K aktiviteleri ölçülmüştür. Tarımsal alanlardan toplanan sebze örneklerinde ^{40}K aktivite konsantrasyonu 491.62 Bqkg^{-1} ile 2324.51 Bqkg^{-1} değerleri arasında ve ^{232}Th aktivite konsantrasyonu 15.96 Bqkg^{-1} ile 52.80 Bqkg^{-1} değerleri arasında ve ^{232}Th aktivite konsantrasyonu minimum dedekte edilebilir seviye (MDA) ile 10.54 Bqkg^{-1} arasında olduğu bildirilmiştir. Sebze örneklerinin toplandığı tarımsal alanlardaki toprak örneklerinde ^{40}K, ^{226}Ra,

^{232}Th aktivite konsantrasyonları sırasıyla 325.89-530.52 Bqkg^{-1}, 46.5-68.83 Bqkg^{-1}, 9.29-50.57 Bqkg^{-1} aralıklarında bulunmuştur. Gübre kullanılmayan alanlardaki toprak örnekleri için aktivite konsantrasyonları; ^{40}K için 240.40-403.09 Bqkg^{-1} aralığında, ^{226}Ra için 35.61 Bqkg^{-1} aralığında ve ^{232}Th için 7.40-38.53 Bqkg^{-1} aralığında bulunmuştur. Çalışmada fosfat gübreleri kullanımının topraklardaki doğal radyonüklidlerin aktivite konsantrasyonlarını az da olsa değiştirdiği bildirilmiştir (Bolca ve ark., 2007).

Bu çalışmanın amacı Kars ilinin Sarıkamış ilçesi ve çevresinden toplanan buğday unu örneklerinin doğal radyoaktivite seviyelerini belirlemektir. Toprak ve buğday unu örneklerinin ^{226}Ra, ^{232}Th ve ^{40}K aktivite değerleri NaI(Tl) sintilasyon dedektörü kullanılarak tespit edilmiştir. Ayrıca, bu doğal radyonüklidlerin topraktan-bitkiye transfer faktörleri (TF) tespit edilmiştir.

2. MATERYAL VE METOT

Bu çalışmada, ölçüm sahası olarak Kars ilinin Sarıkamış ilçesi ve çevresi seçilmiştir. Yöre halkının günlük hayatta özellikle ekmek yapımında kullandıkları buğday unu örnekleri, 9 farklı istasyonda her biri en az 2'şer kg olacak şekilde çalışma bölgesindeki evlerden temin edilmiştir. Buğday unu örneklerinin toplandığı istasyonlar Şekil 1'de verilmektedir.

Toplanan tüm örnekler naylon torbalara konmuş ve torbalar etiketlenmiştir. Daha sonra toplanan örnekler laboratuvara getirilmiştir. Laboratuvarda örnekler elekten geçirilerek ve 105C^{o} de 24 saat süreyle etüvde kurutulmuştur. Daha sonra numuneleri, 100 ml hacimli, 65x55 mm ebatlı, darası alınmış vida kapaklı şeffaf patolojik numune kaplarına yerleştirilip hassas teraziyle tartılarak, ağırlıkları kilogram cinsinden kaydedilmiştir. Örneklerde bulunan; Toryum, Radyum ve bunların bozunma ürünlerinin dengeye gelmesini sağlamak amacıyla kapların ağız kısmı hava geçirmeyecek şekilde bantlanmış ve örnekler kırk günlük süreyle bekletilmiştir.

Şekil 1. Toprak ve buğday unu örneklerinin alındığı istasyonlar

İncelenen örneklerin ^{226}Ra, ^{232}Th, ^{40}K ve ^{137}Cs aktivite konsantrasyonları, NaI(Tl) sintilasyon dedektörlü gama ışını spektrometresi kullanılarak ölçülmüştür. Gama spektrometresinin enerji kalibrasyonu ve verim kalibrasyonu, standart (IAEA-375) kalibrasyon malzemesi kullanılarak gerçekleştirilmiştir. Her bir numune istatistiksel belirsizlikleri azaltabilmek için 86000 saniye sayılmıştır. Net pik alanını hesaplanırken çevreden gelecek katkıların hesaplanabilmesini sağlamak amacıyla, aynı şartlar altında doğal fon ölçümü ayrıca gerçekleştirilmiştir.

Örneklerde bulunan ^{226}Ra aktivite konsantrasyonları hesaplanırken, ^{238}U'in bozunum serisi içinde yer alan ^{214}Bi'un bollukları sırasıyla %46, %15 ve %16 olan 609 keV, 1120 keV, ve 1764 keV'deki piklerinden yararlanılmıştır. Benzer şekilde, ^{208}Tl'e ait bollukları sırasıyla %30.7 ve %35.6 olan 583 ve 2614.5 keV enerjideki pikler ^{232}Th'nin aktivite konsantrasyonunun tayininde kullanılmıştır. Toprakta oldukça yüksek konsantrasyonlarda bulunan ^{40}K aktivite konsantrasyonu ise 1460.8 keV gama piki kullanılarak belirlenmiştir.

Topraktan-bitkiye transfer faktörleri, toprakta ve gıdalarda radyoaktivitenin varlığından dolayı çevresel etkinin değerlendirilmesinde temel öneme sahiptir. Transfer faktörü (TF), bir bitkinin, üzerinde büyüdüğü topraktan aktivite alma veya biriktirme kabiliyetinin indeksidir. Topraktan-bitkiye transfer faktörü ile ilgili çevresel radyoaktivite ölçümlerinde temel olarak doğal radyonüklidler incelenmektedir (Khan Hasan ve ark., 2010). Topraktan-bitkiye transfer faktörü genellikle bir radyonüklidin; bitki örneklerindeki aktivite konsantrasyonunun

topraktaki aktivite konsantrasyonuna oranı olarak tanımlanır. Transfer faktörü aşağıda verilen (1) denklemi ile hesaplanır (Bilgici Cengiz, 2019).

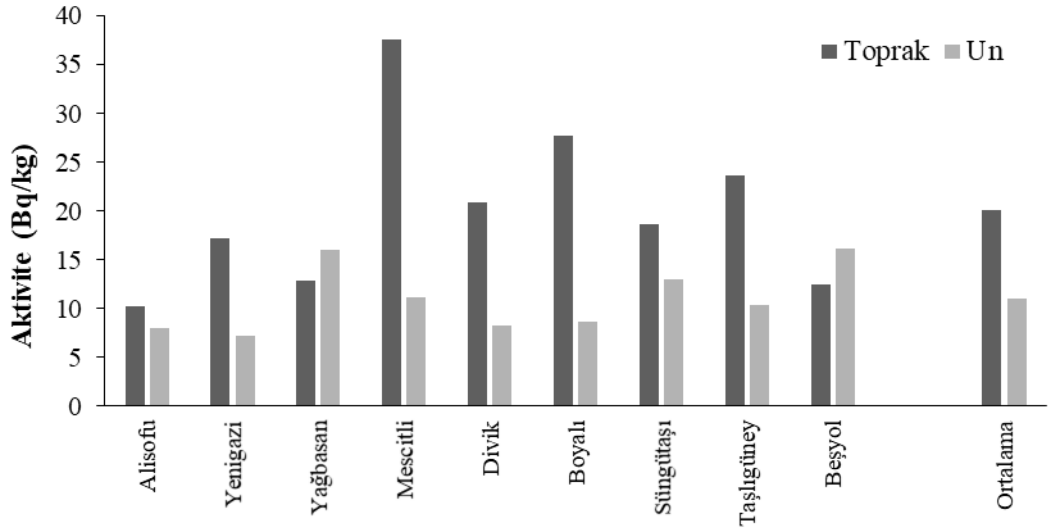
$$TF = \frac{A_{Bitki}}{A_{Toprak}} \quad (1)$$

Denklem (1)'de A_{Bitki} ve A_{Toprak} Bqkg^{-1} biriminde sırasıyla, bitki ve toprak örneklerinin ortalama aktivite konsantrasyonlarıdır. Transfer faktörü değerleri, çalışma alanı içerisinde topraktaki doğal radyonüklidlerin ne kadarının bitkilere aktarıldığını değerlendirmek amacıyla kullanılmaktadır (Al-Hamarneh ve ark., 2016; Bilgici Cengiz ve Cağlar, 2018).

3. BULGULAR

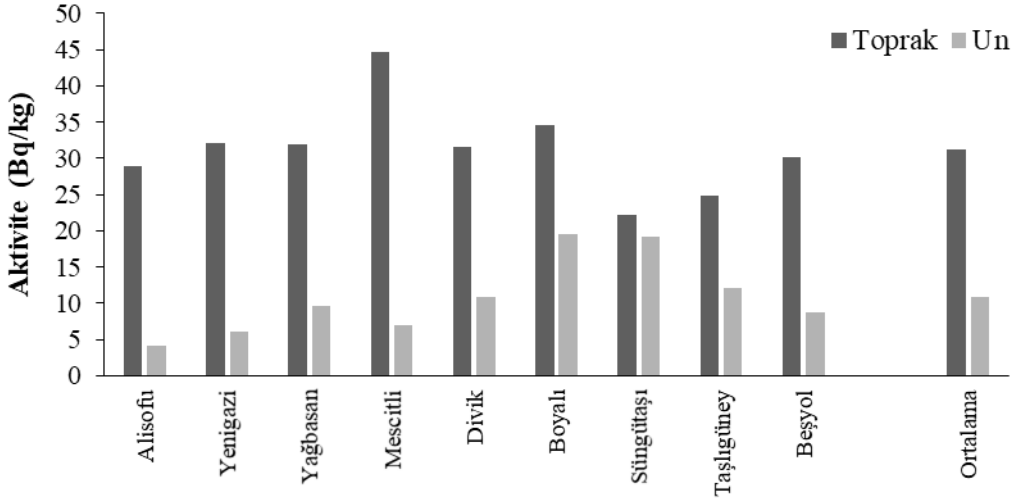
Çalışma bölgesinde yetiştirilen buğday un haline dönüştürülerek yöre halkı tarafından ağırlıklı olarak ekmek yapımında kullanılmaktadır. Bu nedenle insanlar tarafından tüketilen buğdayın doğal radyoaktivite seviyelerinin belirlenmesi, insan sağlığı açısından büyük öneme sahiptir. Ayrıca bölgede yetiştirilen buğday ve diğer yem bitkileri küçük ve büyükbaş hayvan besiciliğinde yaygın olarak kullanılmaktadır. Bu hayvanlardan elde edilen et, süt, peynir, yumurta gibi besinlerin hem yöre halkı hem de ülkemizin farklı bölgelerinde yaşayan insanlar tarafından beslenme amaçlı tüketilmesi nedeniyle çevre sağlığı açısından incelenmesi faydalı olacaktır. Bu bağlamda çalışma bölgesinden toplanan toprak örneklerinin ve yerel çiftçilerden temin edilen bu topraklarda yetiştirilmiş buğday unu örneklerinin ^{226}Ra, ^{232}Th ve ^{40}K aktivite konsantrasyonları ölçülmüş ve değerler Tablo 1'de verilmiştir.

İncelenen toprak ve buğday unu örneklerinde ^{226}Ra ortalama aktivite konsantrasyonu sırasıyla, 20.1±8.2 ve 11.0±2.2 Bqkg^{-1} olarak bulunmuştur. Şekil 2'de ^{226}Ra aktivite konsantrasyonlarının çalışma istasyonlarına göre dağılımı verilmektedir. İncelenen toprak örneklerindeki en düşük ^{226}Ra aktivite konsantrasyonu Alisofu köyünde (10.2±2.0 Bqkg^{-1}) en yüksek ^{226}Ra aktivite konsantrasyonu ise Mescitli köyünde (37.6±8.5 Bqkg^{-1}) gözlemlenmiştir. Buğday unu örneklerinin ^{226}Ra aktivite konsantrasyonunun minimum değeri 7.2±0.9 Bqkg^{-1} ve maksimum değeri 16.1±2.6 Bqkg^{-1} olarak ölçülmüştür.



Şekil 2. Toprak ve Buğday unu örneklerinde ^{226}Ra aktivite konsantrasyonu

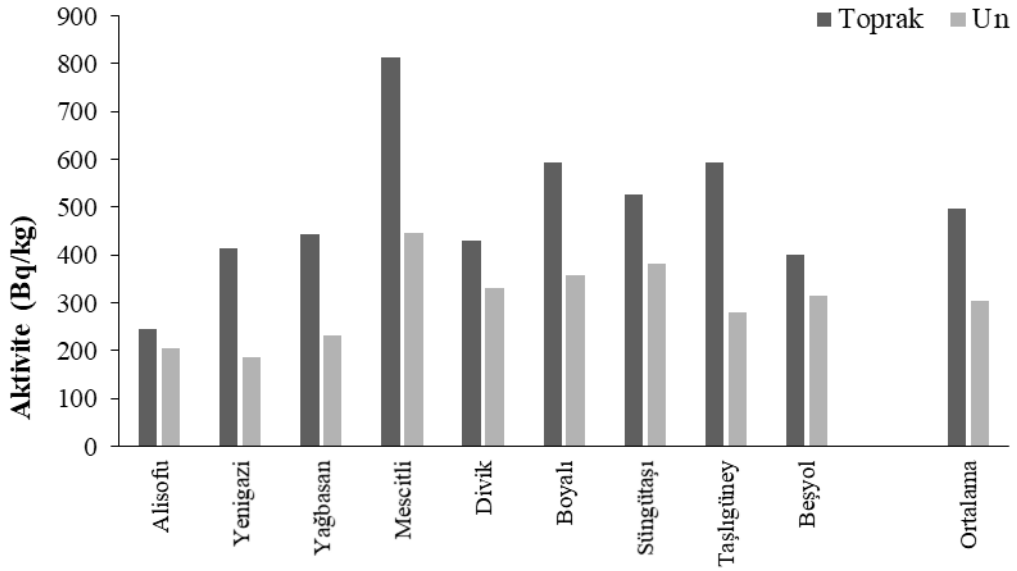
Çalıştığımız toprak örneklerinde, ^{232}Th'nin aktivite konsantrasyonu 22.2±6.8 Bqkg^{-1} ile 44.6±7.5 Bqkg^{-1} değerleri arasında değişmekle birlikte ortalama 31.2±7.1 Bqkg^{-1} olarak tespit edilmiştir. İncelenen buğday unu örneklerinde ^{232}Th ortalama aktivite konsantrasyonu sırasıyla, 10.8±2.3 Bqkg^{-1} olarak bulunmuştur. ^{232}Th aktivite konsantrasyonu en düşük değeri 4.2±1.9 Bqkg^{-1}, en yüksek değeri ise 19.5±2.0 Bqkg^{-1} olarak ölçülmüştür. Şekil 3'te toprak ve buğday unu örneklerindeki ^{232}Th aktivite konsantrasyonlarının çalışma istasyonlarına göre dağılımı verilmektedir.



Şekil 3. Toprak ve Buğday unu örneklerinde ^{232}Th Aktivite Konsantrasyonları

Toprak örneklerindeki ^{40}K aktivite konsantrasyonu; 245.6±34.6 Bqkg^{-1} ile 814.2±35.7 Bqkg^{-1} değerleri arasında ortalama 496.1±35.6 Bqkg^{-1} olarak tespit edilmiştir. İncelenen

buğday unu örneklerinde ^{40}K ortalama aktivite konsantrasyonu ise 304.1±25.5 $Bqkg^{-1}$ olarak bulunmuştur. ^{40}K aktivite konsantrasyonu en düşük değeri 185.7±26.0, en yüksek değer 446.1±27.0 $Bqkg^{-1}$ olarak ölçülmüştür. Şekil 4'te ^{40}K aktivite konsantrasyonlarının çalışma istasyonlarına göre dağılımı verilmektedir.



Şekil 4. Toprak ve Buğday unu örneklerinde ^{40}K Aktivite Konsantrasyonları

Şekil 4'ten de görüldüğü gibi en düşük ^{40}K aktivite konsantrasyonu Yenigazi köyünden alınan örneklerde, en yüksek ^{40}K aktivite konsantrasyonu ise Mescitli köyünden alınan örneklerde ölçülmüştür. İncelenen toprak ve buğday unu örneklerindeki ^{226}Ra, ^{232}Th ve ^{40}K aktivite konsantrasyonları Tablo 1'de verilmektedir

Tablo 1. Toprak ve buğday unu örneklerinde ^{226}Ra, ^{232}Th ve ^{40}K aktivite konsantrasyonları

İstasyon Adı	Aktivite (Bqkg^{-1})					
	^{226}Ra		**^{32}Th**		**^{40}K**	
	Toprak	Un	Toprak	Un	Toprak	Un
Alısofu	10.2±2.0	8.0±1.3	29.0±7.2	4.2±1.9	245.6±34.6	204.1±24.5
Yenigazi	17.2±9.7	7.2±0.9	32.1±7.8	6.0±5.1	414.1±37.7	185.7±26.0
Yağbasan	12.9±9.6	16.0±2.8	31.97.4	9.7±1.9	444.1±37.8	232.8±26.1
Mescitli	37.6±8.5	11.2±2.9	44.6±7.5	7.0±2.5	814.2±35.7	446.1±27.0
Divik	20.9±8.8	8.2±1.4	31.5±6.8	10.9±1.9	429.2±34.2	331.3±25.2
Boyalı	27.7±9.8	8.6±3.0	34.6±7.5	19.5±2.0	594.7±38.9	357.8±26.5
Süngütaşı	18.7±8.4	13.0±2.9	22.2±6.8	19.1±2.3	527.8±33.6	381.9±27.1
Taşlıgüney	23.6±8.1	10.4±2.5	24.8±6.5	12.1±1.7	594.1±32.9	281.5±22.9
Beşyol	12.5±8.9	16.1±2.6	30.2±6.7	8.7±1.8	400.4±34.8	315.4±24.6
Ortalama	20.1±8.2	11.0±2.2	31.2±7.1	10.8±2.3	496.1±35.6	304.1±25.5

Türkiye Atom Enerjisi Kurumunun (TAEK) 2010 yılı raporunda, ^{226}Ra, ^{232}Th ve ^{40}K ortalama aktivite konsantrasyonlarının Türkiye ortalamaları sırasıyla, 34.7±1.7 Bqkg^{-1} 35.4±0.8 Bqkg^{-1} ve 450.0±18 Bqkg^{-1} olarak rapor edilmiştir (TAEA, 2010). ^{226}Ra, ^{232}Th ve ^{40}K ortalama aktivite konsantrasyonlarının Dünya ortalamaları ise UNSCEAR 2000 raporunda sırasıyla 35.0 Bqkg^{-1}, 30.0 Bqkg^{-1} ve 400.0 Bqkg^{-1} olarak rapor edilmiştir (UNSCEAR, 2000). Ölçümlerden elde ettiğimiz sonuçlarla karşılaştırıldığında, çalışma bölgesinde ölçülen ^{40}K ortalama aktivite konsantrasyonu Türkiye ortalamasının biraz altında Dünya ortalamasının ise biraz üzerindedir. ^{226}Ra ve ^{232}Th ortalama aktivite konsantrasyonları ise Türkiye ve Dünya ortalamalarının altındadır. Bunların yanı sıra, çalışma bölgesinde ölçülen aktivite konsantrasyonlarının Ülkemizin ve dünyanın farklı bölgelerinde yapılan çalışmalarla uyumlu olduğu görülmektedir (Abu Samreh ve ark., 2014; Alzubaidi ve ark., 2016; Bilgici Cengiz, 2017; Bilgici Cengiz ve Çağlar, 2016; 2018; Bilgici Cengiz ve Öztanrıöver, 2018; Cengiz ve Reşitoğlu, 2014; Chandrasekaran ve ark., 2014; Dizman ve ark., 2010; Kapdan ve ark., 2011; Karataşlı ve ark., 2016; Oyeyemi ve ark., 2017; Rafique ve ark., 2014; Taşkın ve ark., 2009).

Tablo 2'de çalışma bölgesinden toplanan buğday ununa ait doğal radyoaktivite düzeylerinin ortalama değerlerinin literatürde bazı çalışmalarda bildirilen değerlerle ve aynı spektrometre ile yapılan çalışmayla karşılaştırılması verilmektedir. Kars ilinin Digor ilçesinde yapılan çalışmada mera bitkileri için bildirilen ^{226}Ra, ^{232}Th ve ^{40}K ortalama aktivitelerinin buğday unu için elde ettiğimiz ortalama aktivite değerlerinden daha yüksek olduğu görülmektedir (Bilgici Cengiz 2019). Gediz havzasında yapılan çalışmada mısır örnekleri için ^{226}Ra ve ^{40}K aktivite konsantrasyonları sırasıyla 25.82 ± 2.3 Bqkg^{-1} ve 4491.62 ± 52.61 Bqkg^{-}

[1], Batı Anadolu bölgesinde incelenen lahana örnekleri için ^{232}Th ve ^{40}K aktivite konsantrasyonları 45.5±5.2 Bqkg^{-1} ve 766.0±40 Bqkg^{-1} olarak rapor edilmiş olup incelediğimiz buğday örnekleri için elde ettiğimiz değerlerin bu değerlerin altındadır (Bolca ve ark., 2007; Topcuoğlu ve ark., 2003). Tablo 2'den görülebileceği gibi incelediğimiz buğday unu örnekleri için elde ettiğimiz ortalama ^{232}Th ortalama aktivite değeri market çaylarındaki ^{232}Th ortalama aktivite değerinden biraz üzerinde ve lahana örnekleri için rapor edilen ^{232}Th ortalama aktivite değerinden daha küçüktür. İncelediğimiz buğday unu örneklerindeki ^{40}K ortalama aktivitesi ise mısır, lahana ve market çaylarındaki ^{40}K ortalama aktivite değerlerinden daha küçüktür(Bolca ve ark., 2007; Topcuoğlu ve ark., 2003; Kılıç ve ark., 2009). Dünyanın farklı bölgelerinden yapılan çalışmalarla kıyasladığımızda, buğday unu örnekleri için elde ettiğimiz ortalama ^{226}Ra, ^{232}Th ve ^{40}K aktivite değerleri, Hindistan ve Pakistan için bildirilen buğday örneklerindeki ve Arabistan'da hurma örnekleri için bildirilen aktivite değerlerinden biraz daha yüksektir(Pulhani ve ark., 2005; Khan Hasan ve ark., 2010; Abu Shayeb ve ark., 2018). Bunun yanı sıra, ^{232}Th ortalama Sırbistan'da tıbbi bitkiler için rapor edilen değerden biraz büyük, ^{226}Ra ve ^{40}K aktivite değerleri ise daha küçüktür (Djelic ve ark., 2016).

Tablo 2. Bitki örneklerinin doğal radyoaktivite düzeylerinin ortalama değerlerinin literatürde bildirilen değerlerle karşılaştırılması.

Referanslar	Çalışılan Bölge	Çalışılan Bitki	Aktivite (Bqkg^{-1})		
			^{226}Ra	^{232}Th	^{40}K
Bu çalışma	Sarıkamış	Buğday unu	11.0 ±2.2	11.0 ±2.2	304.1 ±25.5
Bilgici Cengiz, 2019	Digor	Mera bitkisi	17.9±10.4	75.9±19.5	630.6±12.3
Bolca ve ark., 2007	Gediz	Mısır	25.82± 2.34		491.62 ±52.61
Topcuoğlu ve ark., 2003	Batı Anadolu	Lahana		45.5±5.2	766.0±40
Kılıç ve ark., 2009	Türkiye	Market Çayı		5.9±1.7	766.0±40
Pulhani ve ark., 2005	Hindistan	Buğday	0.7±0.1	1.1±0.02	102.9±9.8
Abu Shayeb ve ark., 2018	Suudi Arabistan	Hurma	5.6±1.2	2.8±0.4	181±17
Djelic ve ark. 2016	Sırbistan	Tıbbı Bitki	14.6	9.87	647
Ballesteros ve ark., 2015	İspanya	Arpa			78.0–222.4
Ballesteros ve ark., 2015	İspanya	Buğday			67.0–122.6
Ballesteros ve ark., 2015	İspanya	Mısır			45–118
Khan Hasan ve ark., 2010	Pakistan	Buğday	3.7	8.4	130.7

Transfer faktörü değerleri, çalışma alanı içerisinde topraktaki doğal radyonüklidlerin ne kadarının bitkilere aktarıldığını değerlendirmek amacıyla kullanılmaktadır (Al-Hamarneh ve ark. 2016). Çalıştığımız buğday unu örneklerinde ^{226}Ra radyonüklidi için transfer faktörü 0.30±0.10 ile 1.29±0.95 değerleri arasında ortalama 0.65±0.37 olarak bulunmuştur. ^{232}Th radyonüklidi için transfer faktörü 0.16±0.05 ile 0.86±0.28 değerleri arasında ortalama

0.37±0.13 olarak bulunmuştur. ^{40}K radyonüklidi için ise transfer faktörü 0.45±0.07 ile 0.83 ±0.15 değerleri arasında ortalama 0.63±0.08 olarak bulunmuştur. Çalışma bölgesinden toplanan buğday unu örnekleri için hesaplanan transfer faktörleri ve literatürde bildirilen bazı çalışmaların sonuçlarıyla Tablo 3'de karşılaştırılmış ve bu çalışmada^{226}Ra, ^{232}Th ve ^{40}K için hesapladığımız transfer faktörlerinin literatürde bildirilen çalışmalardaki değerlerle uyumlu olduğu görülmüştür.

Tablo 3: Transfer faktörlerinin değerlendirilmesi

Referanslar	**Çalışılan Bölge**	**Çalışılan Bitki**	**Transfer Faktörleri**		
			^{226}Ra	**^{232}Th**	**^{40}K**
Bu çalışma	Sarıkamış	Buğday Unu	0.30-1.29	0.15-0.86	0.45-0.83
Bilgici Cengiz, 2019	Digor	Mera bitkisi	0.40 ± 0.20	1.44 ± 0.30	0.99 ± 0.023
Pulhani ve ark., 2005	Hindistan	Buğday	0.015	0.019	0.23
Abu Shayeb ve ark., 2018	Suudi Arabistan	Hurma	0.33	0.22	0.51
Djelic ve ark. 2016	Sırbistan	Tıbbı Bitki	0.632	0.320	1.76
Khan Hasan ve ark., 2010	Pakistan	Buğday	0.05	0.13	0.17
Al-Hamarneh ve ark. 2016	Suudi Arabistan	Buğday	0.09		
Jazzar ve Thabayneh, 2014	Filistin	Ot	1.26	1.15	1.20

4. TARTIŞMA VE SONUÇ

Bu çalışmada Kars ilinin Sarıkamış ilçesi ve çevresinden toplanan toprak ve buğday unu örneklerinde ^{226}Ra, ^{232}Th ve ^{40}K aktivite seviyeleri ve ayrıca topraktan- buğday ununa transfer faktörleri NaI(Tl) sintilasyon dedektörü kullanılarak belirlenmiştir. Ölçülen ^{226}Ra, ^{232}Th ve ^{40}K aktivite konsantrasyonlarının Türkiye ve Dünyada yapılan bezer çalışmalarla bildirilen değerleri ile uyumlu olduğu görülmüştür.

Ayrıca incelenen un numuneleri için ^{226}Ra, ^{232}Th ve ^{40}K doğal radyonüklidlerinin topraktan-bitkiye transfer faktörlerinin sırasıyla, 0.30-1.29, 0.15-0.86 ve 0.45-0.83 aralıklarında değiştiği görülmüştür. İncelenen buğday unu örneklerinde elde edilen transfer faktörü değerlerinin literatürdeki benzer çalışmalarda bildirilen transfer faktörleri değerlerinden kısmen farklı olmasının nedeni, buğday bitkilerinin yetiştiği toprağın organik madde içeriği, buğday bitkisinin tohum çeşitliliğine ve bölgenin jeolojik yapısı ile açıklanabilir.

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Investigation of the Effect of Acrylamide on *Capoeta Capoeta* (Guldensttead 1773) by Histopathological, Electrophoretic and Biochemical Methods

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Abstract: The aim of this study was to investigate the effects of acrylamide on *Capoeta capoeta* (Guldensttead 1773) by histopathological, electrophoretic and biochemical methods. *Capoeta capoeta* caught from Kars stream were used in the study. The fish were divided into 5 groups, each containing 10 pieces, and placed in 300 liter tanks with tap water. Group 1 was kept as a negative control. 20 mg / kg cyclophosphamide given to group 2 (i.p. positive control group), 10 mg/L acrylamide given to group 3, 20 mg/L acrylamide given to group 4 and 30 mg/L acrylamide given to group 5. After all groups were kept in tanks for 4 days, blood and tissue samples taken from fish were investigated by histopathological, electrophoretic and biochemical methods. As a result of the analyzes serum AST and ALT levels were decreased in the other groups compared to the negative control group and serum TAS levels were significantly increased in the 30 mg/L acrylamide group compared to the negative control group ($P < 0.01$). Compared with the negative control group, TOS levels were increased in all groups. When the electropherogram obtained from SDS-PAGE was examined, it was determined increases and decreases at 21 kD, 27 kD, 36 kD, 42 kD, 48 kD, 54 kD protein expressions in groups with different concentrations compared to the negative control group. It was observed that protein expressions were inhibited especially in the group treated with 20 mg/L acrylamide. As a result of histopathological examinations; increased degenerations were detected in the gill and liver tissues of fish due to the concentration of acrylamide. As a result; acrylamide treatment caused toxic effects on *C. capoeta* after this varying time intervals and concentrations.

Keywords: Acrylamide, *Capoeta capoeta*, fish, Protein expression, AST, ALT, Histopathology.

1. INTRODUCTION

Acrylamide is crystalline, colorless, odorless, and soluble in water, acetone, methanol, and ethanol. The open molecule formula is C_3H_5NO (CH_2 = CH-$CONH_2$). Acrylamide is

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referred to as, propenamide, ethylene carboxamide, acrylic amide and vinyl amide and is used in polyacrylamide synthesis. Polyacrylamide is preferred in mineral, asphalt, paper production, crude oil processing, drinking and wastewater treatment, soil, and sand purification. It is also used in cosmetic industry, electrophoresis and molecular biology applications, photographic film and adhesive manufacturing, varnish and paint industry, and in the preparation of alloys in dentistry (Iarc, 1994; Europäische Kommission, 2002; Erdogan and Dastan, 2010).

When preparing foods, applications such as cooking, roasting and baking, temperature degrees ranging from 90-200 °C are used. These processes can lead to the formation of toxic compounds in foods. These toxic compounds include heterocyclic amines, polycyclic aromatic hydrocarbons, N-alkyl-N-nitrosamines and acrylamide (Claeys et al., 2005). Acrylamide is absorbed from the digestive tract and passed into the blood after oral administration. It disperses well in the body and crosses the barrier of placenta and passes into the fetus and milk (Tritscher, 2004).

Acrylamide is a substance that causes poisoning and increases the risk of cancer in humans. Acrylamide is in the group of suspected carcinogens in humans (2 / A). It causes cancer in laboratory animals given high concentrations orally. It has the potential carcinogenic in humans. In 2005, WHO and FAO suggested that some foods cooked at high temperatures contain significant levels of acrylamide and their consumption is risky for humans (FAO/WHO, 2012; Zhang et al., 2006).

The determination of acrylamide to be harmful to living things has necessitated a multidimensional approach. As a result of the literature reviews, no information was found about the histopathological, electrophoretic and biochemical effects of acrylamide on *Capoeta capoeta*. In this study it was aimed to investigate the effects of acrylamide on *Capoeta capoeta* by histopathological, electrophoretic and biochemical methods. In this study, the effects of higher levels of acrylamide than acceptable levels in aquatic environments will be revealed.

2. MATERIALS AND METHOD

2.1.Chemicals

Acrylamide and cyclophosphamide were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2.Animals

* The study was conducted with permission from Kafkas University Animal Experiments Local Ethics Committee (KAU - HADYEK: 2014 / 023).

Fifty *Capoeta capoeta*, weighing 200-300 g caught from Kars Stream were used in the research. During the collection of fish, stream water's pH was 8.2-8.4, temperature was 16-18 and dissolved oxygen was 4.52-10.51 mg/L. Low-voltage shock and flip net were used to catch fish. After the fish were caught, they were placed in the water filled from this medium and oxygen-bonded drums. The fish brought to the laboratory were kept in aquariums with tap water for 10 days to adapt to the environment.

2.3. Experimental design

Fish were divided into groups, one containing 10 fish. Test concentrations were determined considering the lower and upper limit ranges used in previous studies (Petersen et al.,1987; Weideborg et al.,2001).

1. Group (n=10): Negative control group. Fish in this group were kept in a tank (300 L) with tap water and no treatment was performed.

2. Group (n=10): Positive control. The fish in this group were kept in a tank (300 L) with tap water and each fish was injected with 20 mg/kg cyclophosphamide intraperitoneally (i.p) (Grisolia et al., 2000).

3. Group (n=10): 10 mg/L concentration of acrylamide was added to 300-liter tanks containing tap water and the fish were kept in this environment.

4. Group (n=10): 20 mg/L concentration of acrylamide was added to 300-liter tanks containing tap water and the fish were kept in this environment.

5. Group (n=10): 30 mg/L concentration of acrylamide was added to 300-liter tanks containing tap water and the fish were kept in this environment.

The following investigations started after 4 days.

2.4. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (Sds-Page) Method

Blood samples were taken from the dorsal veins of the fish and centrifuged at + 4 ℃ and 3000 rpm for 10 minutes to separate the serum and stored at - 20 ℃. Protein concentrations of serum samples were determined by the biuret method (Eisenthal et al., 1992) and SDS-PAGE was performed according to Laemmli and O'Farrell methods

(Laemmli, 1970; O'Farrell,1975). GangNam-Stain ™ Prestained Protein Ladder protein marker was used as the protein standard for electrophoresis process.

2.5. Biochemical Analysis Method

Serum aspartate aminotransferase (AST) (EnzyChrom™ Aspartate Transaminase Assay Kit, USA), alanine aminotransferase (ALT), (EnzyChrom™ Alanine Transaminase Assay Kit, USA), total antioxidant status (TAS) and total oxidant status (TOS) Assay kit (Rel Assay Diagnostics, Clinical Chemistry Solutions, Gaziantep, Turkey) levels, determined spectrophotometrically by the commercial kit (Erel, 2004, Erel, 2005).

2.6. Histopathological Method

Tissue samples taken from the specimens were fixed in 10% formaldehyde and then preparations were prepared by histopathological methods. These preparations were stained according to the hematoxylin-eosin staining method and examined under a light microscope (Luna, 1968).

2.7. Statistical Analysis Method

SPSS 22 package program was used for statistical evaluation of the data obtained from the study. One-way ANOVA was used for statistical analysis of the difference between group data. $P < 0.05$ was considered statistically significant.

3. RESULTS

There were no deaths in the control and experimental groups and no behavioral changes were observed in the samples.

3.1. Electrophoretic and Biochemical Analyzes

Serum AST enzyme levels were significantly decreased in animals treated with 30 mg/L acrylamide and 20 mg / kg cyclophosphamide compared to the negative control group ($P < 0.001$). It was determined that ALT enzyme levels were statistically decreased significantly in all experimental groups compared to the negative control group ($P < 0.001$). The TAS levels of the animals were found to be increased ($P < 0.01$) in the 30 mg/L administered group compared to the negative control group, whereas the increases and decreases in the other groups were not statistically significant. TOS levels were found to be increased in animals in all groups compared to the negative control group. The increase in 20 mg/L acrylamide group was statistically significant ($P < 0.001$) (Table 1).

Table 1: AST, ALT, TAS and TOS levels of the control group and animals treated with different concentrations of acrylamide.

	Control	10 mg/L	20 mg/L	30 mg/L	Cyclophos-phamide	P value
AST (ng/mL)	155 ± 18.3^{a}	134 ± 30.8^{a}	135 ± 22.5^{a}	60 ± 19.7^{b}	60 ± 16.5^{b}	0.001
ALT (ng/mL)	72.8 ± 14.9^{a}	38.5 ± 7.6^{b}	32.9 ± 11.2^{b}	31.1 ± 14.2^{b}	31.4 ± 17.6^{b}	0.001
TAS (mmol Trolox eq/L	4.4 ± 1.0^{bc}	3.5 ± 0.6^{c}	4.9 ± 1.8^{abc}	6.6 ± 1.3^{a}	6.0 ± 2.0^{ab}	0.001
TOS (µmol H_2O_2 eq/L)	28 ± 13.5^{c}	124 ± 25.2^{b}	168 ± 27.0^{a}	116 ± 24.8^{b}	87 ± 20.9^{b}	0.001

* Different letters on the same line indicate statistical significance.

When the electrophoregram obtained from SDS-PAGE was examined, it was found that there were variable increases and decreases in the protein expressions of 21 kD, 27 kD, 36 kD, 42 kD, 48 kD, and 54 kD in acrylamide groups compared to control groups. In particular, protein expressions were inhibited in the group treated with 20 mg/L acrylamide (Figure 1).

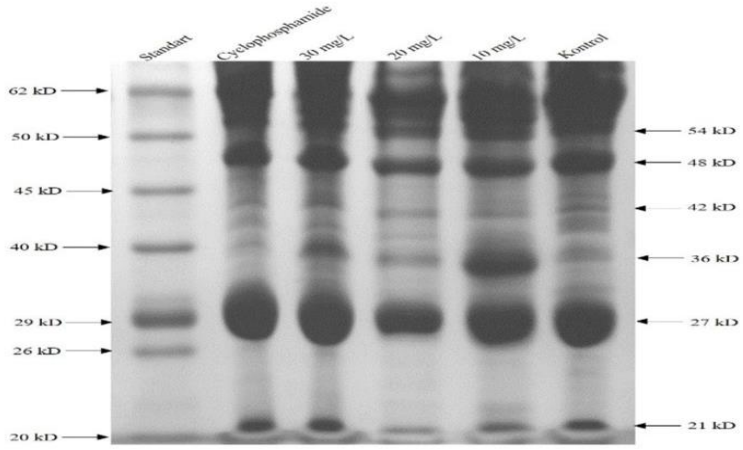
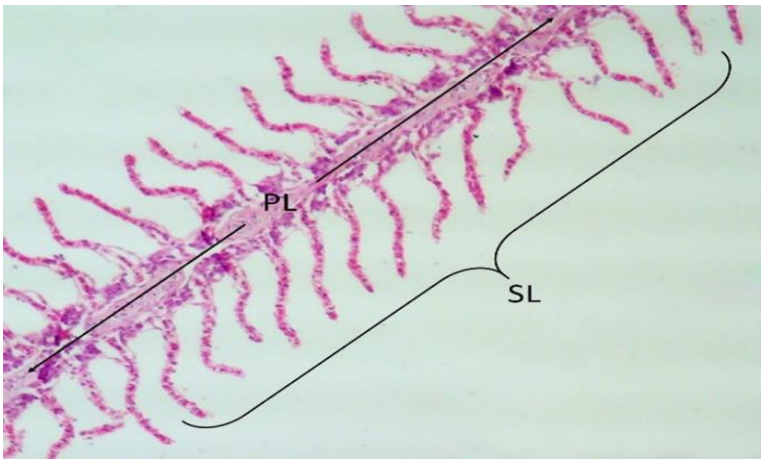


Figure 1: SDS-PAGE electropherogram of the control group and experimental groups.

3.2. Histopathological Analysis

3.2.1. Gills

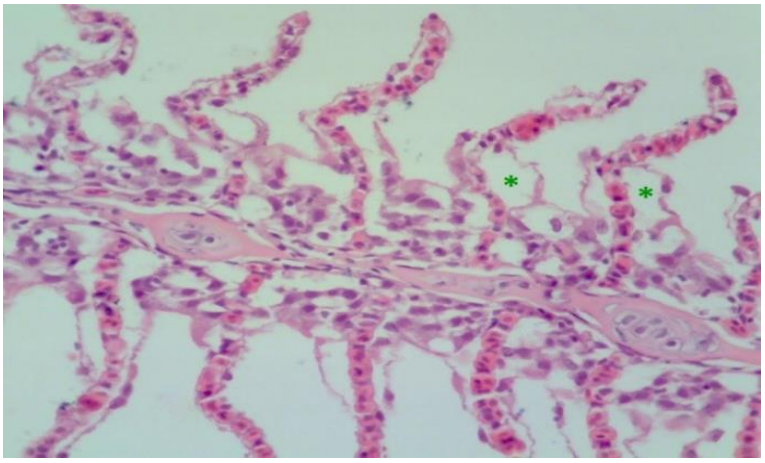
In the *Capoeta capoeta* control group, it was observed that the gill structure consisted of the secondary lamellae, which generally originated from the primary lamellae forming the main axis, and the epithelial covering surrounding the lamellae structure (Figure 2).



PL: primary lamellae, SL: secondary lamellae (H&E).

Figure 2. *Capoeta capoeta* control group, gill histology.

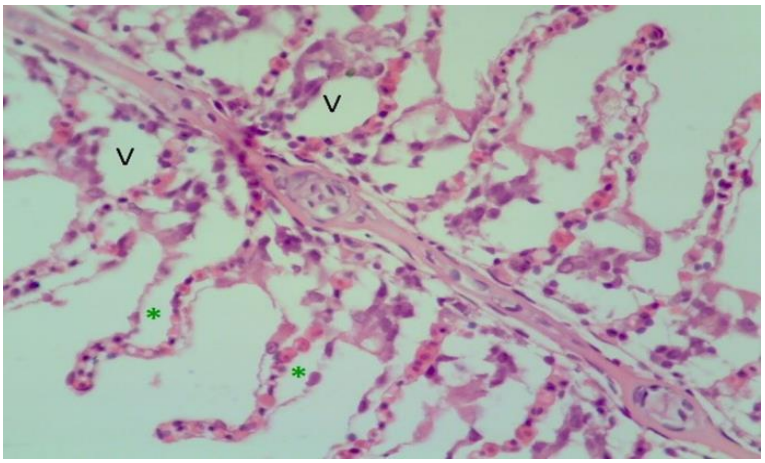
In the 10 mg/L concentrations acrylamide group, the gill structure was similar to the negative control group, but mild edema was detected (Figure 3).



*: edema (H&E).

Figure 3. *C. capoeta* low concentration group, gill histology

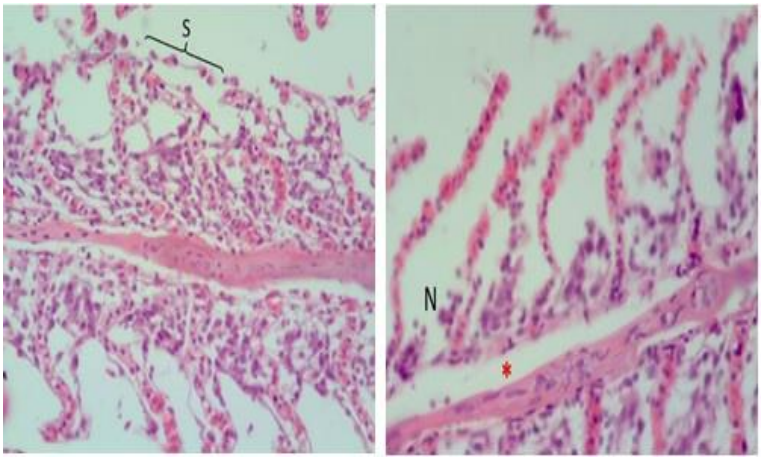
Unlike those exposed to acrylamide at a concentration of 10 mg/L, distinctive vacuolization was detected in the gills of fish given 20 mg/L acrylamide. In addition, edema was detected in the gills (Figure 4).



*: edema, V: vacuolization (H&E).

Figure 4. *C. capoeta* medium concentration group, gill histology.

In groups exposed to concentrations of 30 mg/L of acrylamide, the general appearance of secondary lamellae in the gill structure was irregular and epithelial separation was also determined. The significant separation was observed between the main axis of the cartilage of the primary lamellae and the epithelial layer. The presence of significant necrosis and deterioration of overall tissue integrity were detected in the epithelial layer covering the primary lamellae (Figures 5).



S: epithelial separation; *: separation, N: necrosis (H&E).

Figure. 5. *C. capoeta* high concentration group gill histology.

3.2.2. Liver

According to the observations in the negative control group, there was no lobular arrangement around the central vein in the *C. capoeta* liver parenchyma and series of hepatocytes lined around the central vein were seen with very thin sinusoids and bile ducts

located between them. Polygonal-shaped and large nucleus hepatocytes, which are the main cells of the parenchyma, were found to form fairly smooth cords (Figures 6a-b).

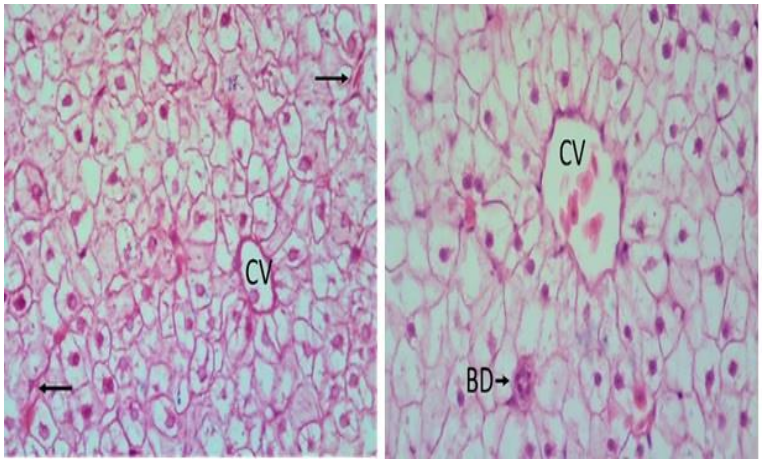


Figure 6a. *Capoeta capoeta* control group, liver histology. CV: central vein, arrows: sinusoids, **6b.** CV: central vein, BD: bile duct (H&E).

In the low concentration group (10 mg/L acrylamide) fish liver histology, sinusoid and central vein vasodilatation and increase in the number of erythrocytes, as well as the increased number of melanomacrophages to form clusters in the parenchyma was found to be the most prominent change (Figure 7).

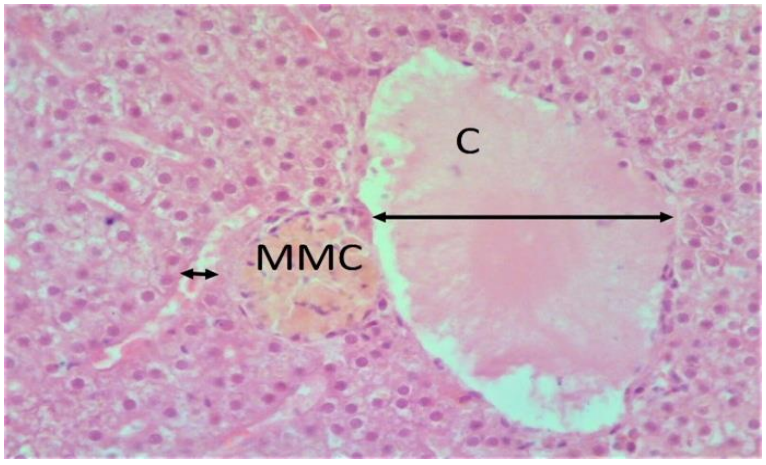


Figure 7. *C. capoeta* low concentration group, liver histology. ↔: vasodilatation, C: congestion, MMC: melanomacrophage cluster (H&E).

Acrylamide exposure to moderate concentration (20 mg/L acrylamide) revealed necrosis in addition to nucleic anomalies such as the pyknotic nucleus, karyorrhexic nucleus and karyolytic nucleus in hepatocytes in the liver parenchyma (Figures 8a-b).

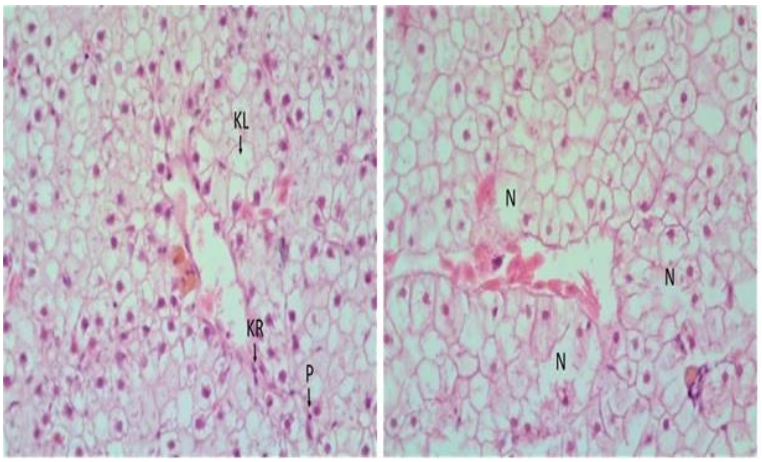


Figure 8a. *C. capoeta* medium concentration group, liver histology. P: pyknotic nucleus, KR: karyorrhexic nucleus, KL: karyolytic nucleus, **8b.** N: necrosis (H&E).

In the high concentration group (30 mg/L acrylamide), the incidence of necrosis in the liver parenchyma was found to increase and create large voids. Fibrosis was observed in hepatocytes within the parenchyma. Also, blood leakage into the parenchyma was detected due to deformations in the vessel walls (Figures 9a-b).

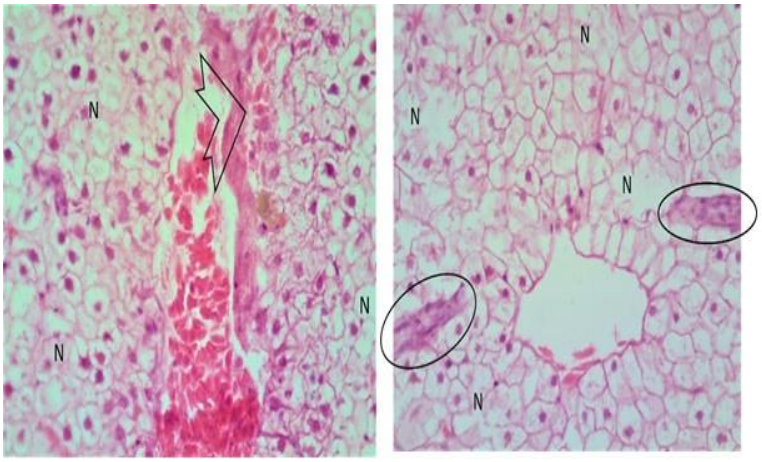


Figure 9a. *C. capoeta* high concentration group, liver histology.
⇨ : blood leakage into the parenchyma, N: necrosis, **9b.** Ellipse: fibrosis, N: necrosis (H&E).

4. DISCUSSION AND CONCLUSION

Experimental studies on various animals have shown that acrylamide is carcinogen and mutagen. Acylamide is also a possible carcinogen and mutagen in humans (Besaratinia et al., 2005).

Polyacrylamide is used in the production of paper, the composition of cosmetic products and the molecular biology laboratory (Manière et al., 2005). It was determined that acrylamide formed

spontaneously during high-temperature cooking of carbohydrate-rich foods (Stadler et al., 2002). The fact that it is taken into the body in various proportions by the food has increased the interest in acrylamide. In a study, glycidamide, considered to be the main metabolite of acrylamide, has been reported to have more genotoxic effects than acrylamide (Martins et al., 2006).

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) are two liver enzymes involved in glyconeogenesis and amino acid metabolism (Zareei et al., 2017). In particular, skeletal muscle, cardiac muscle, red blood cell, and liver cells carry high concentrations of AST enzyme. ALT is an enzyme found in primarily hepatocyte cytoplasm (Mayer et al., 2013). In the present study, it was determined that AST and ALT enzyme levels decreased in acrylamide treated fish. In fact, it is reported that the levels of these enzymes increase due to cell damage and toxicity due to the treatment of acrylamide (Nagi et al., 2014). HepG2 cells, a hepatocellular carcinoma cell line that maintains many functional properties of normal human hepatocytes, play an important role in determining human hepatocyte function (Mavri-Damelin et al., 2007). It has been reported that intracellular AST activity is inhibited as a result of changes in the structure of these cells due to the introduction of copper complexes into HepG2 cells (Jia et al., 2017). It has been suggested that the decrease in the level of aminotransferase due to acrylamide administration may result from the changes caused by the negative effect of acrylamide on HepG2 cells and/or excessive use of AST and ALT in meeting the energy need in the metabolic struggle against acrylamide toxicity (Larguinho et al., 2014a).

In toxicology studies conducted on different fish species, it was stated that the antioxidant level generally decreased and the oxidant level increased depending on the fish species and the substance applied. Depending on the glyphosate administration on Oncorhynchus mykiss (Nur and Deveci, 2018), on Cyprinus carpio administered with 2 mg / L and 3 mg / L tebuconazole, in the 3 mg / L group (Kaya et al., 2014), on Capoeta capoeta as a result of zinc sulfate application at a dose of 10 mg / L (Deveci et al.2015) and similarly on Capoeta capoeta administered with 0.01 mg / L and 0.02 mg / L glyphosate, in the group treated with 0.02 mg / L (Deveci et al., 2017) it is reported that the level of TAS decreases and the TOS level increases.

Experimental acrylamide toxicity studies reveal different results in terms of oxidative stress. It is stated that the reason for this is due to the different treatment ways and dosage of acrylamide (Çınar, 2010). Acrylamide treatment in rats was found to decrease antioxidant enzyme activity in the cerebral cortex and increase lipid peroxidation (Lakshmi et al., 2012). It was reported that there was no difference in MDA level compared to the control group, and GSH-Px antioxidant enzyme level decreased in rats (Çınar, 2010). It has been found that the treatment of acrylamide in rats increases the MDA level (El-Beltagi et al., 2016). It has been reported that glutathione S-transferase and superoxide dismutase activities increase in rats' plasma, liver, testis, brain, and kidney due to acrylamide treatment (El-Demerdash et al., 2006). It has been suggested that acrylamide causes an increase in MDA and GST enzyme activities in *Mytilus galloprovincialis* species (mussel) (Larguinho et al., 2014b). In this

study, an increase was observed in the TAS level of the animals in the group given only 30 mg/L of acrylamide, while an increase was found in the TOS level of the animals in all groups compared to the control group. It is reported that acrylamide can react with cellular nucleophiles having -SH, NH2 or -OH as well as reacting with GSH and forming glutathione S-conjugates (Awad et al., 1998). It is thought that the increase in TAS level due to acrylamide treatment may result from the formation of glutathione S-conjugates.

As a result of physiological stress, structural and functional changes occur in cellular proteins in living organisms (Shwetha et al., 2012). The change in protein fractions in the organism can be attributed to the deterioration of their structure or their possible overuse (Naveed et al., 2010). Proteins are used in the process of protecting the organism against oxidative stress caused by exposure to acrylamide. Acrylamide causes a significant decrease in the content of sulfhydryl groups and protein contents in different tissues (El-Demerdash et al., 2006). In this study, it was found that changes occurred in different protein expressions at different concentrations applied. An increase in 36 kD protein expression occurred in the group treated with 10 mg/L acrylamide, while protein expressions were inhibited in the group treated with 20 mg/L acrylamide.

Histopathological studies have shown that liver epithelial cells of rats are damaged significantly due to the treatment of acrylamide (Altinoz et al., 2015). Petersen et al. determined that acrylamide administration caused hyperplasia and metaplasia in the gills of the Rainbow trout (distal of secondary lamellae) (Petersen et al., 1987). The results of this research support the results of this study. In another study; Larguinho et al. treated concentrations of 1-10 mg/L of acrylamide to *Mytilus galloprovincialis.* They found that acrylamide has no histopathological effects on the gill (Larguinho et al., 2014b). In another study, it was reported that the liver parenchyma of *Carassius auratus* exposed to acrylamide is almost entirely composed of eosinophilic cells, and necrosis and focal hyperemia are seen) (Larguinho et al., 2014a). The results of these researches are in parallel with this study.

It can be said that the effects of acrylamide increase gradually depending on the concentration administered. This condition was detected in both the gill and liver tissues as a result of histopathological examinations. As a result, it can be suggested that acrylamide disrupts antioxidant/oxidant balance, affects liver enzyme activities and protein expression, and causes structural deterioration in gill and liver tissues in *Capoeta capoeta.*

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