

Investigation of In Vitro Effect and Molecular Docking of Aluminum on Glucose-6-Phosphate Dehydrogenase Activity

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ABSTRACT

Aim: Aluminum is one of the elements that is widely used in many sectors and is the most abundant element in nature. The harm of aluminum, which was thought to be harmless until recently and is actively used in daily life, is open to discussion. In this study, it was aimed to investigate the effect of Aluminum Sulphate [Al₂(SO₄)₃] on Glucose-6-Phosphate Dehydrogenase (G6PDH) activity, which is a key enzyme that catalyzes the first step of the pentose phosphate pathway (PPF). In addition, enzyme activity are detailed with molecular docking studies. For the purpose of examining in vitro effect of Aluminium on G6PDH, 4 different concentration of substrate (D-glucose-6-P) (0.1M, 0.08M, 0.05M, 0.03M) prepared and 10mM, 30mM Al₂(SO₄)₃ was added G6PDH envoriment. G6PDH activity was measured by spectrofotometrically. Molecular docking studies were performed with DockingServer and HEX 8.0.0 programs. With the data obtained, the Vmax of G6PDH was calculated as 3.33 and Km=0.0323. When 10 mM and 30mM Al₂(SO₄)₃ were added to the reaction environment, it was observed that there was a decrease in enzyme activity by 24.92% and 57.06%, respectively. It was observed that the increase in Al₂(SO₄)₃ concentration was an uncompetitive inhibition due to a significant decrease in both Km and Vmax values of the enzyme.

Keywords: Aluminium, Glucose 6-Phosphat dehydrogenase, Uncompetitive inhibition, Enzyme.

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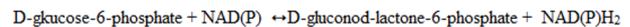
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Introduction

Nowadays, the rapid development of industry, the change in production methods in agriculture (use of artificial fertilizers, pesticides, etc.), the unhealthy storage of solid wastes and wastewater, and the contamination of some heavy minerals used in food storage conditions to living things [1]. Aluminum (Al) is widely used in many industrial fields (electricity, petroleum, chemistry, space, furniture, household), food and pharmaceutical industries. Al is taken into the body through diet, respiration and skin [2,3]. Normally, the accumulation of Al in the body is prevented, but in pathological conditions, the body Al load increases and leads to toxicity [4]. Most of the Al in the body is excreted through the urine. Studies have shown that Al accumulates in tissues such as liver, kidney, brain and lung in renal dysfunction and in some pathological conditions [5,6]. Although Al is one of the most abundant elements in nature, many of its forms are not harmful to living things. However, it turns into harmful derivatives at low pH [7,8]. It is reported that the accumulation of Al and its derivatives in the body plays an important role in the pathogenesis of many diseases, especially Alzheimer's [3,9].

Glucose-6-Phosphate Dehydrogenase (EC 1.1.1.49) is an important enzyme with its coenzyme NADP⁺, which plays a role in the rate-limiting step of PFY [10] and is widely found in all tissues and erythrocyte cells (RBC).



G6PDH enzyme deficiency is the most common hereditary enzyme deficiency in the world [11]. G6PDH deficiency, which is the most common and clinically important enzyme in the world, causes hemolytic anemia [12]. Since changes in this enzyme activity will negatively affect metabolism, it is of vital importance.

Molecular docking process allows to examine the biological activities and enzyme inhibition properties of molecules at the molecular level. With the molecular docking process, the interactions between the minimalized protein structure of cell lines and the drug candidate can be examined at the molecular level. By molecular docking, the binding energies, binding modes and types of secondary chemical interactions between the target protein and the molecule under study can be determined [13].

In this study, it was aimed to investigate the in vitro effect of Al and its derivatives, which are widely used in various pharmaceutical, food, agriculture and many industrial areas, on the vitally important G6PDH enzyme activity. Molecular docking studies are of great importance in cell biology, as they are performed by interacting a target protein or enzyme (cell line) with a ligand molecule. It is a key step in drug development. The docking results can be used to find the inhibitor of the

target protein and the biological activities of the studied chemical species can be studied at the molecular level.

Materials and Methods

Chemicals and Apparatus

Tris-HCL, NADP, G6PDH, D-glucose-6-P were purchased from Sigma Aldrich (Steinheim, Germany), Glycine, $Al_2(SO_4)_3$, $MgCl_2$ were purchased from MERCK (Darmstadt, Germany), BSA was purchased from Amresco (USA). All chemicals used are of analytical purity. All solutions used in this study were prepared with deionized Milli-Q water (Millipore, Bedford, MA, USA). Dual beam path Spectrophotometer (Labmed. Inc) and electronic precision balance (Sartorius 000032) were used. Experimental studies were carried out at room temperature (25.0 ± 0.5) °C.

Measuring G6PDH Activity

Experimental method steps in the Worthington Enzyme Manual [14] were followed to examine the effect of aluminum against the kinetic behavior of the G6PDH enzyme. The activity values against 4 different (0.1, 0.08, 0.05, 0.03 mM) substrate concentrations were calculated and the Lineweaver-Burk plot with $1/Activity$ versus $1/concentration$ plot was drawn. In the presence of $Al_2(SO_4)_3$ with 10 mM and 30 mM concentrations, it was added to each of the 4 different concentrations of substrate medium as inhibitor. Activity values were recorded. Activity experiments were repeated three times. The average of the data obtained here was transferred to the Lineweaver-Burk graph. Maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) of the enzyme were calculated (Table-1). The reaction rate of the G6PDH enzyme was determined by the reduction of absorbance at 340 nm as a result of reduction of NADP. The reduction of $1\mu mol$ NADP per minute at pH 7.8 and 30°C was determined as one unit of activity. The specific activity values of the G6PDH enzyme were calculated with the help of the following equation [14]. With the help of these data, the in vitro effect of $Al_2(SO_4)_3$ on G6PDH activity was evaluated.

$$Units /mg = \frac{\Delta A_{340}/min}{6,22 \times mg \text{ enzyme}/mL \text{ reaction mix}}$$

Molecular Docking Calculation

Docking Server was used as the docking program. For this, the geometry optimization of the ligands was done again with the MMFF94 method. The load calculation method was chosen as Gasteiger. pH = 7.0 was taken for all calculations. In docking calculations, grid maps are $90 \times 90 \times 90 \text{ \AA}$ (x, y and z) and Lamarckian genetic algorithm (LGA) and Solis & wet local search method was used [15]. During docking, the population size was set to 150. A translation step of 0.2 \AA and a 5 \AA quaternion and torsion steps were applied during the search for the appropriate region of the target protein of the molecules studied. HEX

8.0.0 [16] was preferred for the overall exposure of the studied compound.

Results

Lineweaver-Burk graph was drawn according to the data obtained using D-glucose-6-P at 4 different concentrations (0.1, 0.08, 0.05, 0.03 mM). The V_{max} value of G6PDH was calculated as $3.33 /mg \text{ protein}/min$, and the K_m value was calculated as 0.0323 mM (Figure-1). G6PDH activity was measured by adding $Al_2(SO_4)_3$ solution prepared at two different concentrations (10 mM and 30 mM) to the reaction medium. Lineweaver-Burk curve was drawn according to the obtained data (Figure-2). In the presence of 10 mM, the $Al_2(SO_4)_3$ K_m value was calculated as 0.029 and the V_{max} as $2.5 (\mu mol \text{ D-G6P}/mg \text{ protein})/min$. In the presence of 30 mM, the $Al_2(SO_4)_3$ K_m value was calculated as 0.0271 and the V_{max} as $1.43 (\mu mol \text{ D-G6P}/mg \text{ protein})/minute$. It was observed that there was a 24.9% decrease in G6PDH activity in the presence of 10 mM $Al_2(SO_4)_3$ and a 57.06% decrease in the presence of 30 mM $Al_2(SO_4)_3$ (Table 1).

Looking at the data obtained, it was seen that $Al_2(SO_4)_3$ inhibited the G6PDH enzyme uncompetitively.

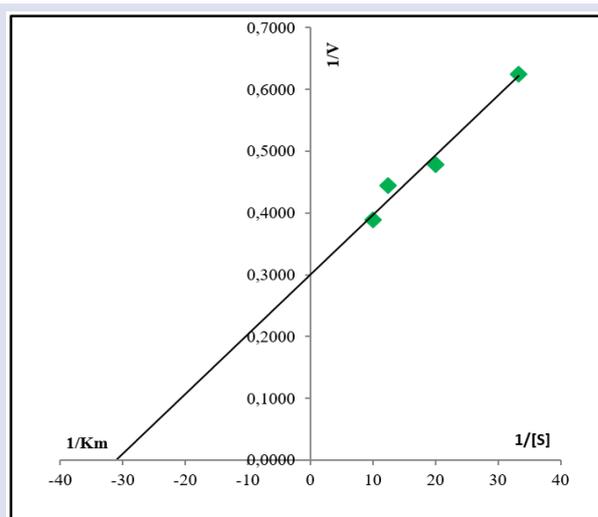


Figure 1. Lineweaver-Burk plot of G6PDH without inhibitor

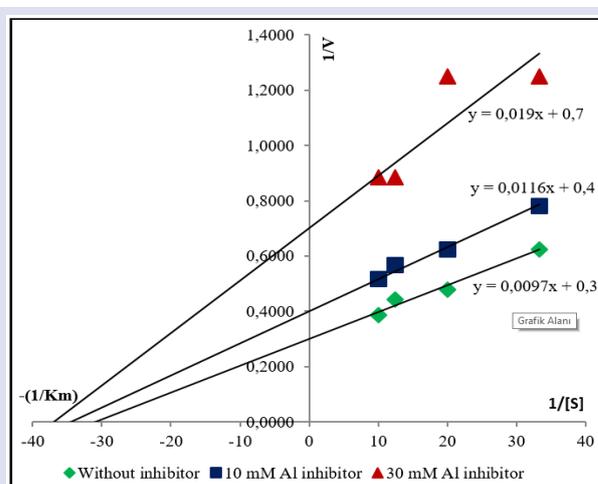


Figure 2. Lineweaver-Burk plot of the effect of $Al_2(SO_4)_3$ on G6PDH enzyme activity

Table 1. Kinetic Properties of G6PDH Enzyme and Al₂(SO₄)₃

	G6PDH Enzyme				
	No Inhibitor	10mM Al ₂ (SO ₄) ₃		30mM Al ₂ (SO ₄) ₃	
K _m (mM)	0.0323	0.029	10.22%	0.0271	16.10%
V _{max} (µmol D-G6P/mg protein/dk.)	3.33	0.0323	24.92%	1.43	57.06%

Molecular docking is of great importance in cell biology because it is done by interacting a target protein with a ligand molecule. It is very important in terms of determining the interactions that take place in biological processes. The docking results can be used to find the

inhibitor of the target protein. Target proteins were determined in the protein database. PDB ID=1DPG [17] target proteins were selected for the glucose 6-phosphate dehydrogenase (G6PDH) enzyme. Geometry optimization for Al₂(SO₄)₃ and selected target proteins was done by MMFF94 method. Docking processes were repeated with both programs. An important parameter used while giving molecular docking results is the binding energy. Binding energy includes interactions such as van der Waals interactions, electrostatic interactions, hydrogen bonds, and hydrophobic interactions. The magnitude of the binding energy is a measure of the stability of the ligand-receptor complex [18]. The affinity of two molecules can be estimated using the binding energy. Interaction types, docking poses and binding modes of Al₂(SO₄)₃ with target proteins representing G6PDH enzyme are given in the Figure 3.

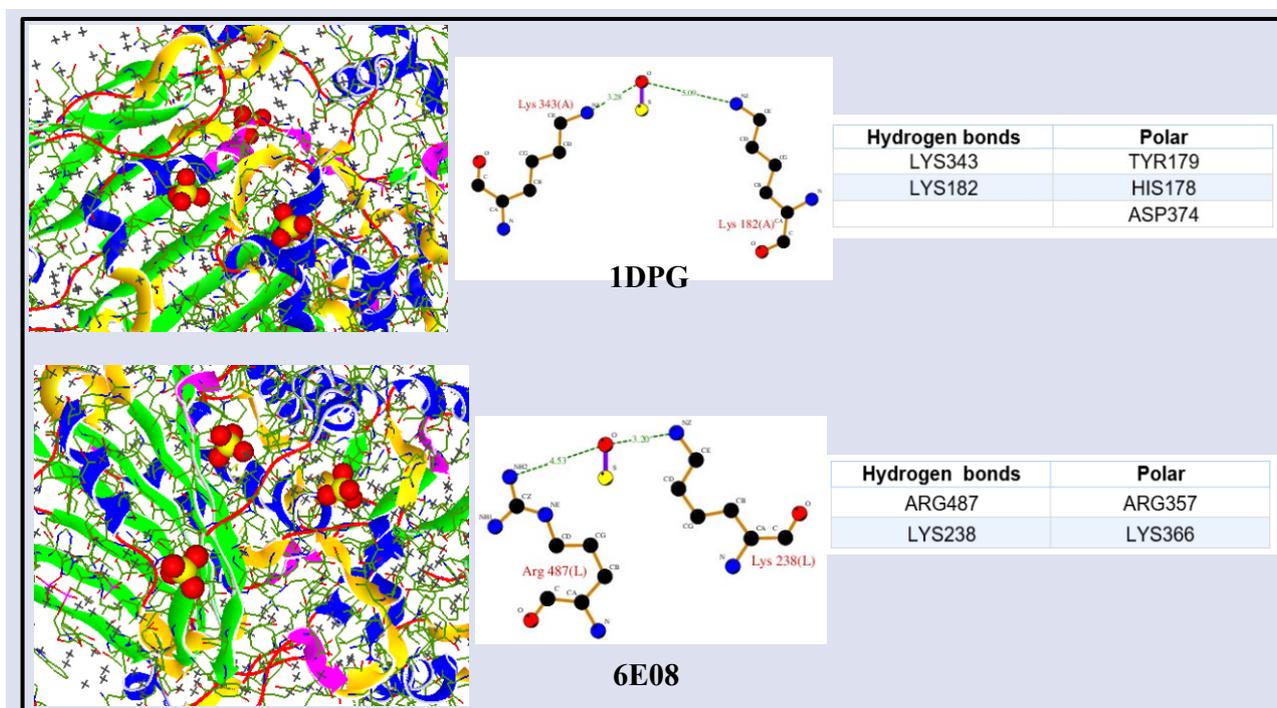


Figure 3. Binding modes, docking poses and interaction types between investigated ligand and target proteins.

When the interaction types between Al₂(SO₄)₃ and the target proteins, whose docking poses are given in Figure 3, are examined, there are H-bond and polar interactions. The studied ligand forms H-bond with amino acid residues LYS343 and LYS182 of the 1DPG target protein. TYR179 is in polar interaction with amino acid residues HIS178 and ASP374. Al₂(SO₄)₃ ligand 6E08 forms an H-bond with amino acid residues ARG487, LYS238 and polar interaction with amino acid residues ARG357, LYS366 of the target protein. The binding energy (BE), intermolecular energy (IE), van der Waals interactions, vdW + Hbond + desolved Energy (WHDE), interaction surface (IS) and inhibition constant (K_i) values to estimate enzyme inhibition activity between ligand-target proteins are given in Table 2.

Table 2. Docking results between Al₂(SO₄)₃ and target proteins

	1DPG	6E08
BE (kcal/mol)	-2.29	-4.93
IE (kcal/mol)	-2.29	-4.93
WHDE (kcal/mol)	-0.82	-1.04
IS	187.645	226.149
K _i (µM)	210.80	245.03

As seen in Table 2, negative values of BE, IE and WHDE energies provide inhibition of both 1DPG and 6E08 enzymes of Al₂(SO₄)₃ at molecular level. More negative interaction energy indicates higher inhibition efficiency [19]. According to the calculated docking results here, considering the energies, it is thought that Al₂(SO₄)₃

inhibits a small molecule AG1, which corrects it in the 6E08 target protein, more since there is no drug available to treat G6PDH deficiency. On the other hand, when the interaction surfaces (IS) of $Al_2(SO_4)_3$ with target proteins are examined, the interactions with both target proteins are almost close. The high interaction surface also increases the ligand-protein interaction and causes an increase in enzyme inhibition activity [20]. The inhibition constant (K_i) is a data about the amount of drug to be used in the treatment. The smaller this value, the smaller the amount of drug used in the treatment [21]. In addition, the results of the two docking programs show that only sulfur and oxygen atoms interact with amino acid residues. However, it is remarkable that it is included in the target protein in the form of a compound.

Discussion

Erythrocytes are among the most abundant cell types in an adult human, with a gross volume of 2 L and accounting for ~10% of the total cell number. The lifespan of erythrocytes is about 100-120 days, meaning that more than 200 billion erythrocytes containing about 20 mL of filled cell volume need to be replaced every day [22]. Eryptosis can occur due to various cellular stresses such as osmotic shock, oxidative stress and energy deficiency [23]. Oxidative stress or insufficient antioxidative defense activates Ca^{2+} and Cl^- channels, causing the concentration of these intracellular ions to change [24]. In addition, oxidative stress causes eryptosis by causing the activation of aspartyl and cysteinyl proteases in erythrocytes [25]. In erythrocytes, NADPH+H is synthesized in PFY, the only source, in the rate-limiting step catalyzed by the G6PDH enzyme. Necessary for the production of reduced glutathione (GSH), which is important for protection against oxidative damage in NADPH. Fico et al. clearly stated the role of G6PDH in protection from apoptosis and necrosis induced by redox imbalance [26]. In our study, we found that $Al_2(SO_4)_3$ caused uncompetitive inhibition of the G6PDH enzyme.

G6PDH will cause activation of the apoptotic pathway not only for erythrocytes but also within PBMC.

Many researchers have investigated the activator or inhibitory effects of heavy metals on the enzyme system in living organisms [28,29,30]. Although aluminum is not in the heavy metals group, it has been defined as a potentially toxic element in recent years [31]. Although most forms of aluminum are not harmful to living things, they tend to form harmful derivatives at low pH's [32]. Tolerable levels of Al determined by 'The Joint FAO/WHO Expert Committee on Food Additives (JECFA)' for human body is 2 mg/kg/week [33]. Aluminum salts (such as aluminum hydroxide, phosphate, carbonate) are also used for treatment in the field of medicine such as humoral immunity, peptic ulcer and dialysis [34-37]. Several studies have been conducted on aluminum exposure. For example, it is a controversial issue whether the role of aluminum in Alzheimer's disease is due to Al accumulation in the patient's brain or due to Al-induced oxidative stress [38], in a study in rats, $Al_2(SO_4)_3$ was found to cause germinative damage and renal degeneration [39]. Again, in an experimental animal study, it was shown that Al causes anemia with the damage it causes to the hematological system [40]. Sucralfate is an aluminum-containing drug recently approved by the Food and Drug Administration (FDA) for the short-term (up to eight week) treatment of duodenal peptic ulcers. This drug was used in different doses and durations for therapeutic purposes, and it was found that the serum Al levels of the patients increased after the treatment [41]. Aluminum especially can affect important biomolecules of metabolism such as AMP, ATP, ADP, inositol phosphate, Glucose-6-Phosphate, 2,3-diphosphoglycerate. In the relationship between ATP and Mg^{2+} , which takes place in most biological reactions, Mg^{2+} is irreversibly replaced and forms a more stable complex. In addition, it can decrease Ca^{2+} ATPase activity, disrupt intracellular Ca^{2+} movement and inhibit many enzymes [42]. It is known that deficiency or inhibition of G6PDH, the most common and clinically important enzyme in the world, causes hemolytic anemia [12]. NADPH+H produced in the pentose phosphate pathway is required to reduce erythrocytes oxidized (GSSG) glutathione to reduced glutathione (GSH). Changes in G6PDH enzyme activity will cause hemolysis due to increased GSSG level as NADPH+H production will be affected significantly [12.]

In this study, it was determined that aluminum sulfate is an uncompetitive inhibitor of $Al_2(SO_4)_3$ G6PDH enzyme and affects both K_m and V_{max} levels (Figure-2). It is thought that as a result of the increase of aluminum in tissues and blood circulation, G6PDH enzyme will be inhibited and erythrocytes will be affected the most. Insufficient functioning of this enzyme means slowing of PFY and incomplete production of NADPH+H in the cell. The only production pathway of NADPH+H in erythrocytes is the pentose phosphate pathway. Oxidized glutathione (GSSH) is reduced to reduced glutathione (GSH) by the enzyme glutathione reductase, whose coenzyme is NADPH+H. H_2O_2 's occurring in living organisms are

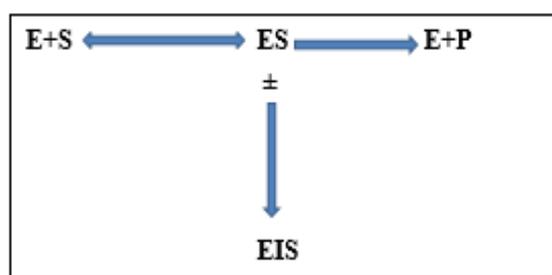


Figure 4. Uncompetitive Enzyme Inhibition (E:Enzyme S:Substrate I:Inhibitor P:Protein)

It is thought that exposure to Al or its compounds for a long time will cause triggering of eryptosis due to the increase in blood concentration. They showed that ROS-producing agents affect peripheral mononuclear cells (PBMC) of patients with G6PDH deficiency, leading to apoptosis due to insufficient protection from oxidative damage by low glutathione [27]. Therefore, inhibition of

converted to water by the enzymes catalase (CAT) and glutathione peroxidase (GPx). GPx needs reduced glutathione (GSH) to perform this reaction. In this case, there is not enough NADPH+H for the antioxidant enzymes involved in the reduction of free radicals to work efficiently [43]. Free radicals, especially reactive oxygen species (ROS), are compounds that can react very actively. It is known that ROT interacts with lipid, protein and DNA, disrupting the structure of these biomolecules and causing many metabolic diseases.

In our study, aluminum sulfate was found to be an uncompetitive inhibitor of the G6PDH enzyme. Mainly aluminum; It is known to bind to phosphate, carboxylate, amines, amino acids, nucleic acids and nucleotides [42]. We believe that the G6PDH enzyme inhibits by binding to these molecules in its structure. As a result of this inhibition, it will not be possible to eliminate free radicals since NADPH+H cannot be produced by pentose phosphate. This will cause hemolysis due to the deterioration of the structure of many biomolecules and damage to cell membranes.

As a result, when the data of our study is evaluated, it is seen that aluminum salts have negative effects on the antioxidant defense system. Accordingly, we think that it is important for people with low plasma G6PDH enzyme levels not to use aluminum-containing food and industrial products if possible, and to inform their physicians if they are given aluminum-containing drugs. With molecular docking studies, for the first time, the glucose-6-phosphate dehydrogenase (G6PDH) enzyme inhibition of $Al_2(SO_4)_3$ was investigated at the molecular level and its inhibition efficiency was theoretically supported.

Conflicts of Interest

The authors state that did not have conflict of interests

References

- [1] Yılmaz E., Farklı Dozlardaki Alüminyum Klorür'ün Galleria Mellonella (Lepidoptera: pyralidae)'nın Biyolojisine ve Hemosit Sayılarına Etkileri (Doctoral dissertation), Marmara Üniversitesi, (2013).
- [2] Ak Uysal H., Ergene N.B., Alüminyum ve İnsan Sağlığı, *SÜ Tıp Fakültesi Dergisi*, 6(2) (1990) 230-237.
- [3] Yenigül E., Alüminyum ve Bazı Organik Asitlerin Etkileşiminin Spektrometrik ve Kromatografik Yöntemlerle Tayini, (2006).
- [4] Alfrey A.C., Aluminum, *Advances in Clinical Chemistry*, 23 (1983) 69-91.
- [5] Ojeda C. B., Rojas F.S., Pavón J.C., Torres A.G., Automated Online Separation Preconcentration System For Platinum Determination by Electrothermal Atomic Absorption Spectrometry, *Analytica Chimica Acta*, 494 (1-2) (2003) 97-103.
- [6] Akpolat T., Utaş C., Hemodiyaliz Hekimi El Kitabı, Kayseri, Anadolu Yayıncılık (2001).
- [7] Becaria A., Campbell A., Bondy S. C., Aluminum as a Toxicant, *Toxicology and Industrial Health*, 18(7) (2002) 309-320.
- [8] Haberhauer G., Ja A.A., Tunega D., Gerzabek Martin H., Lischka H., Potential of Theoretical Calculations to Estimate the Stability of Al^{3+} /Citrate, Oxalate and Acetate Complexes, In 17. World congress of Soil Science, Bangkok (Thailand), August (2002).
- [9] Savory J., Opening of the Mitochondrial Permeability Transition Pore by Reactive Oxygen Species is a Basic Event Neurodegeneration, Virginia Univ Charlottesville, (2001).
- [10] Telefoncu A., Telefoncu F., Glukoz-6-fosfat Dehidrogenaz Aktivitesine Primaquine'nin Etkisi, *Tr. J. Medical Sci.*, 14 (1989) 57-63.
- [11] Greer J. P., Arber D.A., Glader B., List A.F., Means R.T., Paraskevas F., Rodgers G.M., Wintrobe's Clinical Hematology, Lippincott Williams & Wilkins (2013).
- [12] Sodeman W. A., TM., Sodeman's Pathologic Physiology Mechanisms of Disease, 1 (1992).
- [13] Kaya S., Erkan S., Karakaş D., Computational Investigation of Molecular Structures, Spectroscopic Properties and Antitumor-Antibacterial Activities of Some Schiff Bases. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 244 (2021) 118829.
- [14] Von Worthington, Worthington Enzyme Manual; Enzymes And Related Biochemicals, 1.1.1.49 Glucose-6-Phosphate Dehydrogenase, (1993) 189-190.
- [15] Bikadi Z., Hazai E., Application of the PM6 Semi-empirical Method to Modeling Proteins Enhances Docking Accuracy of AutoDock, *Journal of Cheminformatics*, 1(1) (2009) 1-16.
- [16] Ritchie D., Orpailleur T., Hex 8.0. 0 User Manual. Protein Docking Using Spherical Polar Fourier Correlations Copyright c (2013).
- [17] Rowland P., Basak A.K., Gover S., Levy H.R., Adams M.J., The Three Dimensional Structure of Glucose 6-Phosphate Dehydrogenase from Leuconostoc Mesenteroides Refined At 2.0 Å Resolution, *Structure*, 2(11) (1994) 1073-1087.
- [18] Erkan S., Activity of the Rocuronium Molecule and its Derivatives: A Theoretical Calculation, *Journal of Molecular Structure*, 1189 (2019) 257-264.
- [19] Erkan S., Structural, Spectroscopic and Anti-Cancer Properties of Hydroxy-and Sulfonamide-Azobenzene Platinum (II) Complexes: DFT and Molecular Docking Studies, *Cumhuriyet Science Journal*, 39(4) (2018) 1036-1051.
- [20] Erkan S., Kaya S., Sayin K., Karakaş D., Structural, Spectral Characterization and Molecular Docking Analyses of Mer-Ruthenium (II) Complexes Containing the Bidentate Chelating Ligands, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 224 (2020) 117399.
- [21] Merugu R., Neerudu U.K., Dasa K., Singh K.V., Molecular Docking Studies of Deacetyl-bisacodyl with Intestinal Sucrase-Maltase Enzyme, *Int. J. Adv. Sci. Res.*, 2(12) (2016) 191-193.
- [22] Bosman G., Willekens F., Werre J., Erythrocyte Aging: a More Than Superficial Resemblance to Apoptosis, *Cellular Physiology and Biochemistry*, 16(1-3) (2005) 1-8.
- [23] Lang K. S., Duranton C., Poehlmann H., Myssina S., Bauer C., Lang F., Huber S.M., Cation Channels Trigger Apoptotic Death of Erythrocytes, *Cell Death & Differentiation*, 10(2) (2003) 249-256.
- [24] Duranton C., Huber S. M., Lang F., Oxidation Induces a Cl-Dependent Cation Conductance in Human Red Blood Cells, *The Journal of Physiology*, 539(3) (2002) 847-855.
- [25] Matarrese P., Straface E., Pietraforte D., Gambardella L., Vona R., Maccaglia A., Malorni W., Peroxynitrite Induces Senescence and Apoptosis of Red Blood Cells Through the Activation of Aspartyl and Cysteiny Proteases, *The FASEB Journal*, 19(3) (2005) 1-27.

- [26] Fico A., Paglialonga F., Cigliano L., Abrescia P., Verde P., Martini G., Filosa S., Glucose-6-Phosphate Dehydrogenase Plays a Crucial Role in Protection From Redox-Stress-Induced Apoptosis, *Cell Death & Differentiation*, 11(8) (2004) 823-831.
- [27] Spitz D.R., Azzam E.I., Jian Li J., Gius D., Metabolic Oxidation/Reduction Reactions and Cellular Responses to Ionizing Radiation: a Unifying Concept in Stress Response Biology, *Cancer and Metastasis Reviews*, 23(3) (2004) 311-322.
- [28] Fairbanks V.F., Copper Sulfate-induced Hemolytic Anemia: Inhibition of Glucose-6-Phosphate Dehydrogenase and Other Possible Etiologic Mechanisms, *Archives of Internal Medicine*, 120(4) (1967) 428-432.
- [29] Danielyan K. E., Babayan L. A., Chailyan S. G., Impact of the Heavy Metals on the Activity of Xanthine Oxidoreductase, *Appl Clin Pharmacol Toxicol*, 3(123) (2019) 2577-0225.
- [30] Budak H., Ceylan H., Kocpinar E.F., Gonul N., Erdogan O., Expression of Glucose-6-Phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase in Oxidative Stress Induced by Long-Term Iron Toxicity in Rat Liver, *Journal of Biochemical and Molecular Toxicology*, 28(5) (2014) 217-223.
- [31] Exley C., Korchazhkina O.V., Promotion of formation of Amyloid Fibrils By Aluminium Adenosine Triphosphate (AlATP), *Journal of Inorganic Biochemistry*, 84(3-4) (2001) 215-224.
- [32] Merian E., Anke M., Ihnat M., Stoepler M., Elements and Their Compounds in the Environment, Occurrence, Analysis and Biological Relevance 2nd ed. Wiley-VCH Verlag GmbH and Co. KGaA (2004). (<https://www.cabdirect.org/cabdirect/abstract/20043118056>).
- [33] Serencam H., Arslaner A., Müge K., Civil Peynirde Ağır Metal Kontaminasyon Kaynağı ve Düzeyinin Belirlenmesi Üzerine Bir Araştırma, *Engineering Sciences*, 13(1) (2018) 21-26.
- [34] Onur E. Alüminyum Toksikitesinin Kalite Kontrol Açısından Değerlendirilmesi, *Türk Nefroloji Diyaliz ve Transplantasyon Dergisi*, 1(2) (1997).
- [35] Recker R.R., Blotcky A.J., Leffler J.A., Rack, E.P., Evidence of Aluminum Absorption from the Gastrointestinal Tract and Bone Deposition by Aluminum Carbonate Ingestion with Normal Renal Function, *The Journal of Laboratory and Clinical Medicine*, 90(5) (1977) 810-815.
- [36] Clapp T., Siebert P., Chen D., Braun L.J., Vaccines with Aluminum-Containing Adjuvants: Optimizing Vaccine Efficacy and Thermal Stability, *Journal of Pharmaceutical Sciences*, 100(2) (2011) 388-401.
- [37] Klaassen C.D. (Ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons. New York: McGraw-Hill, 1236 (2013) 189.
- [38] Bakar C., Baba A., Metaller ve İnsan Sağlığı: Yirminci Yüzyıldan Bugüne ve Geleceğe Miras Kalan Çevre Sağlığı Sorunu, 1. Tıbbi Jeoloji Çalıştayı, (2009).
- [39] Kutlubay R., Oğuz E.O., Güven C., Can B., Sinik Z., Tuncay Ö.L., Histological and Ultrastructural Evidence for Protective Effects on Aluminium-induced Kidney Damage by Intraperitoneal Administration of α -Tocopherol, *International Journal of Toxicology*, 26(2) (2007) 95-101.
- [40] Oğuz E. O., Yüksel H., Havane E., Yaşar E., Zorbozan O., Zuhul C., Turgut G., Alüminyum Sülfat'ın "Ross" Cinsi Term Besi Civivi Karaciğerinde Yarattığı Toksik Ve İnflamatuar Hasar, *Ankara Üniversitesi Tıp Fakültesi Mecmuası*, 61(1) (2008) 1-6.
- [41] Pai S., Melethil S., Cuddy P., Hall T., Elevation of Serum Aluminum in Humans on a two-day Sucralfate Regimen, *The Journal of Clinical Pharmacology*, 27(3) (1987) 213-215.
- [42] Günaydin N., Uzun Süreli Alüminyum Kaplarda Yapılan Yoğurtlarla Beslenenlerde Plazma Alüminyum Seviyeleri ile Oksidatif Durum Arasındaki İlişkinin Araştırılması (Master thesis), Sağlık Bilimleri Enstitüsü, (2005).
- [43] Fridovich I., The Biology of Oxygen Radicals: The Superoxide Radical is an Agent of Oxygen Toxicity; Superoxide Dismutases Provide an Important Defense, *Science*, 201(4359) (1978) 875-880.