Uluslararası Fen Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Science Research 2023, Vol. 7 (1), 37-46
https://doi.org/10.29329/ijiasr.2023.524.4

Original article

Effect of L-Dopa on Carboplatin Induced Hepatotoxicity

Çağatay Oltulu [a, *] & Nebiye Pelin Türker [b]

[a] Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye
[b] Technology Research Development Application and Research Center, Trakya University, Edirne, Türkiye

Abstract

Carboplatin is an alkylating antineoplastic drug. Although carboplatin induces apoptosis in rapidly dividing cells like cisplatin, it has fewer side effects. The use of platinum compounds as antineoplastic agents at high doses causes hepatotoxicity, which is important in dose regulation. Levodopa is used as a precursor for dopamine synthesis in the brain in the treatment of Parkinson's disease. Dopamine synthesized in the liver from levodopa cannot cross the blood-brain barrier. There are studies suggesting that dopamine may have a protective effect on hepatocytes. In our study, we aimed to evaluate the cytoprotective effect of levodopa in cell damage induced by carboplatin in the liver cell line. Cell viability was evaluated by MTT test by exposing AML-12 hepatocyte cells to the combination of Carboplatin, L-DOPA and Carboplatin + L-DOPA for 24 hours (0, 3.13, 6.25, 12.5, 25, 50 ,100 µM) has been left. The LD_{50} value of carboplatin was determined as 62.65 µM. The LD_{50} values of the combination of L-DOPA and carboplatin + L-DOPA were higher in the tested dose range. The cytotoxic effect of carboplatin on the AML-12 cell line was determined by the increasing LD_{50} value, which was statistically significantly reduced when combined with levodopa. Further studies are needed to explain the mechanism of action.

Keywords: Liver, L-DOPA, Carboplatin, Cytotoxicity.

Received: 06 August 2022 * **Accepted:** 27 February 2023 * **DOI:** https://doi.org/10.29329/ijiasr.2023.524.4

* **Corresponding author:**

Oltulu Çağatay is an assistant professor in the Department of Pharmaceutical Toxicology at Trakya University in Edirne, Turkey. His research interests include the Toxicology and cytotoxicity. He is living, working, and studying in Edirne, Türkiye.
Email: cagatayo@trakya.edu.tr

INTRODUCTION

Chemotherapy is treatment with anticancer drugs to control the proliferation of cancer cells or to kill cancer cells. Alkylating agents, antimetabolites, antimitotics, antibiotics, monoclonal antibodies, protein kinase inhibitors, selective estrogen modulators, topoisomerase inhibitors are used in chemotherapy. Multiple drug applications are also used in the treatment. Although the scope of chemotherapy drugs is wide, the adverse effects are similar. Chemotherapy drugs can cause central and peripheral neurotoxic effects (1). Neurotoxic side effects are most common effect in the treatment of platinum compounds cisplatin and oxaliplatin. It has been suggested that different mechanisms are responsible for the occurrence of neurotoxicity (2). DNA-protein, DNA inter strand and intra strand adducts formation, DNA base excision repair inhibition mechanisms are one of the mechanisms in neurotoxicity formation. Nonmitotic nerve cells are also affected, and axonal shrinkage formation as a result of DNA damage in sensory neurons is the most critical step in neural damage formation (2). Activation of caspase 3 and caspase 9 through the mitochondrial pathway with p53 activation in cisplatin nerve cells causes apoptosis (2). Mitochondrial damage is also important in neurotoxicity. Cisplatin has the same affinity for nuclear DNA and mitochondrial DNA, and as a result of bonding, the electron transport chain is disrupted, energy production decreases, the formation of free oxygen radicals increases (3). It can contribute to neurotoxicity with reduced antioxidant defenses as a result of the formation of free oxygen radicals. Platinum compounds cause voltage-gated ion channel dysfunction and delay the inactivation of sensory neurons (2). Although carboplatin and oxaliplatin have a nephrotoxic effect, the nephrotoxic effect of cisplatin is more common (2). In the formation of nephrotoxicity, the presence of predisposing factors of preexisting renal failure, hypertension is important. Platinum compounds cause hematologic toxicity, the myelosuppressive effect of carboplatin is more frequent. Oxaliplatin and cisplatin can cause hepatotoxicity as a result of causing sinusoidal dilatation in the liver, obstruction, nodular hyperplasia, decrease in function in the liver, formation of ROS in the cells of the sinusoidal epithelium. (2). As a result of the increase in cytokine levels in the body, it makes healthy liver cells sensitive to cell death (2).

Carboplatin is a platinum compound used in the treatment of cancer. Specifically used as a chemotherapeutic drug to treat ovarian, head & neck, and lung malignancies, carboplatin is an intravenously given platinum coordination complex and alkylating agent. Rates of transitory blood aminotransferase increases and occurrences of clinically obvious liver damage are linked with carboplatin treatment (4, 5). Carboplatin is an alkylating chemotherapeutic agent that induces apoptosis in rapidly dividing cells. By causing cross-links between DNA strands, carboplatin inhibits DNA replication, translation, protein synthesis and triggers apoptosis, which is programmed cell death in cancer cells where the proliferation rate is increased. Like other chemotherapy agents, carboplatin causes the formation of free oxygen radicals in cells, depending on exposure time and dose (6, 7). The liver is

the main organ where detoxification reactions occur. Free radicals formed will affect antioxidant levels and will have adverse effects on the liver. Carboplatin induces hepatotoxicity on rats (8). It has been shown that serum glutamic-pyruvic transaminase , alkaline phosphatase, and serum glutamic oxaloacetic transaminase enzyme levels are increased in rats due to hepatotoxicity caused by carboplatin (9). Additionally, large doses of carboplatin have been used in conditioning regimens before hematopoietic cell transplantation, which may be linked to cases of sinusoidal obstruction syndrome, a serious disorder that can result in abrupt liver failure (10). Beginning 10 to 20 days after transplantation, sinusoidal obstruction syndrome often manifests as right upper quadrant discomfort, hepatic soreness, weight gain, edema, and ascites, followed by jaundice (10). It is unclear how carboplatin contributes to these cases of sinusoidal obstruction syndrome (10).

Following chemotherapy treatments using carboplatin and other platinum compounds, specific case reports of hepatitis B reactivation, sinusoidal obstruction syndrome, acute hepatic necrosis, and severe hyperammonemia (without liver damage) have been documented (10). Since carboplatin is often used in combination with other agents, the effects may not be due to carboplatin alone.

Compared to other platinum compound antineoplastic agents, such as cisplatin, carboplatin has fewer side effects. Carboplatin's emetic effect, renal toxicity, ototoxicity, and neurotoxicity are significantly lower than cisplatin's, but its myelosuppressive efficacy is greater. Dose-limiting toxic effect is myelosuppression. Reduces the level of blood cells, especially platelets (11). Hepatotoxicity in high-dose platinum chemotherapy is important in dose regulation (12, 13).

In acute liver injury in mice, dopamine has been reported to exert hepatoprotective effects by suppressing TNFα mRNA expression, suppressing caspase-3 degradation, and reducing the number of TUNEL-positive hepatocytes in hepatocytes (14). It is controversial because the effect of levodopa (L-DOPA) is dose dependent. There are also studies on the cytotoxic effects of levodopa (15). In our study, we aimed to evaluate the cytoprotective effect of L-DOPA on the AML12 liver cell line induced by carboplatin depending on the dose.

MATERIALS and METHODS

Cell Culture:

In our study, we used the mouse AML-12 Hepatocyte cell line with the code CRL-2254 purchased from the American Type Culture Collection (ATCC). Cell line studies were performed at the TUTAGEM facility under sterile conditions. Cells were seeded into flasks with DMEM:EMEM:HAMS F12, 5% newborn bovine serum (FBS), 1% L-glutamine, 10 mg/ml streptomycin, and 100 IU/ml penicillin at 37°C at 95% humidity. It was taken into an oven containing 5% CO2. Adhesion and other properties of the cells were checked at certain intervals with an OLYMPUS brand invert microscope. Proliferated cells were passaged every 3 days.

MTT assay:

AML-12 cells were seeded 180 µL per well. Incubation was done at 37°C for 24 hours in an oven containing 5% CO_2. The MTT test was performed in a 96-well plate. AML-12 cells were seeded in each well in a volume of 180 µL and then incubated at 37°C for 24 hours in an oven with 5% CO_2. 20 µL of the solution containing carboplatin, L-DOPA and their combinations at the concentrations of 0, 3.13, 6.25, 12.5, 25, 50 ,100 µM prepared from the main stock solution dissolved in 0.5% DMSO in distilled water was added to the wells. At the end of the 24-hour incubation period, 20 µL of MTT solution (5 mg/mL) was used on each well and incubated for 2 hours (37°C, 5% CO_2). After two hours, the medium in the wells was removed then 200 µL of DMSO was added to each well. To calculate the % cell viability and the dose at which 50% of the cells died (LD_{50}), the optical densities of the cells were read in the microplate reader device at 492 nm wavelength and calculated with the formula given below.

% Cell Viability:Sample absorbance value/Control absorbance value*100

Statistics:

In our study, the determination of LD_{50} values in the MTT test was made with SPSS 22 (SPSS Inc., Chicago, IL, USA) software with probit analysis (n=4). The viability difference between mean of doses was evaluated with One-way ANOVA test and post hoc Duncan's multiple comparison test ($p<0.05$).

RESULTS and DISCUSSION

Chemotherapeutic drugs used in cancer treatment may cause the development of drug resistance, and they also cause various side effects as a result of their cytotoxic effects on healthy cells. Against these side effects, the use of compounds with proliferative activity can protect against toxicity.

It was proposed that L-dopa, an intermediary in the synthesis of catecholamines, may alter its own metabolism while being administered as a therapy. In a study, it was reported that oral and subcutaneous administration of L-dopa to mice reduced the activity of the liver aromatic L-amino acid decarboxylase enzyme, which catalyzes the conversion of dopa to dopamine, by up to 50%, while the enzyme activity of other organs was not impaired (16). After the final dosage of L-dopa, the liver's decarboxylase activity remained low for at least 5 days.

It is now known that different experimental techniques can alter the activities and quantities of the enzymes that produce norepinephrine (16). In previous studies, it was demonstrated that L-dopa-treated mice had lower tissue levels of the enzymes tyrosine hydroxylase, dopamine-p-hydroxylase and aromatic L-amino-acid decarboxylase were not significantly altered in the previously examined tissues (16). However, multiple studies with patients receiving L-dopa treatment for parkinsonism showed that

this medication may eventually cause changes to its own metabolism, perhaps at the decarboxylation stage. It is understood that ongoing treatment boosts L-therapeutic dopa's effectiveness (16).

The interaction between L-dopa and the cofactor pyridoxal phosphate does not appear to be the mechanism by which liver decarboxylase is affected, and the decrease in enzyme activity is probably the result of a decreased level of enzyme protein. One important pathway for L-dopa metabolism is decarboxylation. Given that the liver is one of the organs with the highest concentrations of dopa-decarboxylase activity, it likely accounts for a large portion of the peripheral metabolism of L-dopa administered to parkinsonism patients. Without a corresponding decline in the brain enzyme, the liver enzyme should gradually decline, increasing the amount of L-dopa accessible to the brain and increasing the amount of norepinephrine and dopamine produced by the central nervous system. These findings are in line with evidence of improved therapeutic effectiveness and a decrease in the required maintenance dosage during protracted L-dopa therapy (17).

The interaction between L-dopa and the cofactor pyridoxal phosphate does not appear to be the mechanism by which liver decarboxylase is affected, and the decrease in enzyme activity is most likely the result of decreased enzyme protein level (16). An important pathway for L-dopa metabolism is decarboxylation. Most of the L-dopa given for the treatment of Parkinson's is converted to dopamine by the high dopamine-decarboxylase activity of the liver and dopamine cannot cross the blood-brain barrier. The decrease in liver decarboxylase activity and the increase in the amount of L-dopa reaching the brain and the increase in dopamine and norepinephrine production with brain decarboxylase enzyme activity are compatible with the decrease in the required maintenance dose during long-term treatment (17).

In a different investigation, L-dopa therapy decreased lymphocyte proliferation and the generation of cytotoxic T cells in response to allogeneic stimulatory cells in vitro. The number of spleen T lymphocytes has decreased as a result of these inhibitory actions. However, L-dopa therapy significantly improved the proliferative response of spleen cells to concanavalin A (18).

In another in-vitro study, it was reported that catecholamines such as L-DOPA, adrenaline and isoproterenol decreased the mitotic index in L-cells (19). The beta-adrenergic antagonist propranolol prevented catecholamines from acting, but the alpha-adrenoblocker phenoxybezamine did not completely stop it. The pretreatment with adrenoblockers had no effect on the reduction in the mitotic index caused by L-DOPA (19). The incorporation of 3H-leucine and 3H-thymidine into the cells' total protein and DNA, respectively, was reduced by catecholamines. Catecholamines could not act on the culture if propranolol was pre-incubated with it for 10–20 minutes before they were administered. Alpha-adrenoreceptor blocker phenoxybenzamine failed to have this effect. The findings show that beta-adrenoreceptors play a major part in the mechanism underlying catecholamines' effects on proliferative processes (19).

In this study, we investigated the protective role of L-DOPA on the toxic effect of Carboplatin in AML-12 healthy liver cells by performing MTT test. We preferred AML-12 cells because they represent healthy hepatocytes and because it is a cell line that is frequently used in studies.

The LD_{50} values of Carboplatin, L-DOPA and their combinations were determined by probit analysis. The LD_{50} dose of carboplatin was found to be 62.5 μM. According to the MTT results shown in Figure 1, the dose and time of administration of carboplatin showed a toxic effect on AML-12 cells. A dose of 62.5 μM in 24 hours was found to be statistically significant ($p<0.001$).

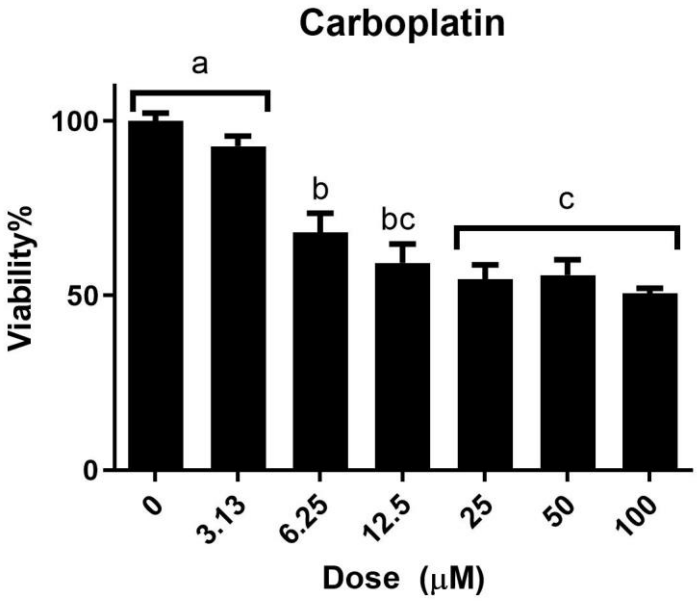

Figure 1. The effect of carboplatin to cell viability. Different letters mean significant difference between treated groups. One-way ANOVA post hoc Duncan (n=4; ± SD; $p< 0.05$).

L-DOPA did not show any toxic effect in AML-12 cells at the dose and hour applied (Fig 2). According to the MTT results shown in Figure 3, combination administration reduced the toxic effect of Carboplatin on AML-12 cells. The LD_{50} dose of the combination group could not be determined statistically at the administered dose and time interval. In the study conducted by Stewart DJ in 2007, it was suggested that it inhibits DNA repair and increases antitumor activity by causing the accumulation of Carboplatin-DNA adducts (20). The use of chemical drugs synthesized for the treatment of cancer cells in patients causes side effects. Therefore, the development of alternative treatment methods and drugs is very important today. However, the use of many chemically synthesized anticancer drugs has caused significant side effects in patients. For this reason, the discovery and development of new drugs based on alternative treatment methods has been the focus of many research. Due to the toxic effect in chemotherapy treatment, combination therapies have been started to be applied. Anticancer and cytotoxic effects on various healthy cells and cancer cells were evaluated in order to develop alternative treatments with some natural origin substances and cancer drugs with less side effects (21, 22).

The toxicity of L-DOPA, which has been used in the treatment of Parkinson's from past to present, and its contribution to neurodegeneration are not fully known. The formation of free oxygen radicals and other reactive oxygen species is based on the autoxidation of L-DOPA and Dopamine. It is thought that this situation destroys the lipid infrastructure of cell membranes as well as cell death. Cell death may have occurred by mitochondrial respiratory chain dysfunction, apoptosis, necrosis and excitotoxicity mechanisms. Studies also show that this toxicity is dependent on the dose of L-DOPA used. Exposure of neuronal cultures to L-DOPA at the doses used increased survival and improved neurite outgrowth of dopaminergic neurons (15, 23). Dopamine is synthesized from L-DOPA in hepatocytes. Dopamine has been shown to have a vasodilatory effect in peripheral tissues through betaadrenoceptors and dopaminergic receptors (24). In another study, it was suggested that the use of dopamine during cardiopulmonary bypass in children with congenital heart disease was effective in order to increase liver perfusion and reduce liver function loss (25).

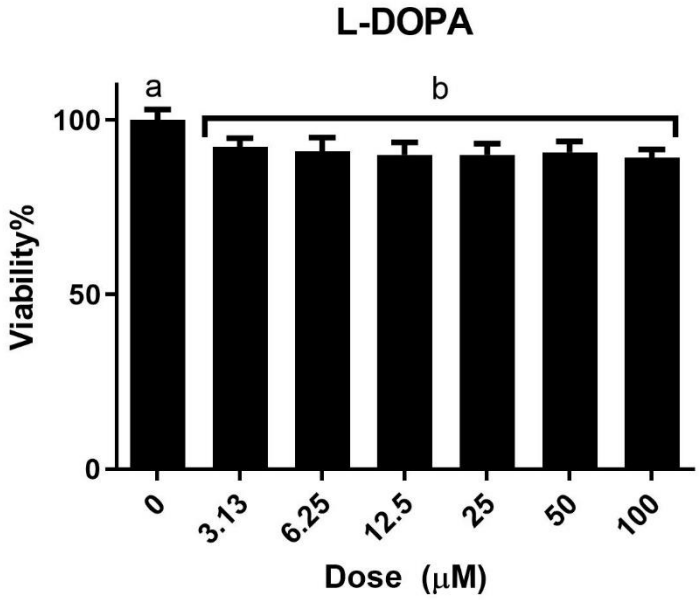

Figure 2. The effect of L-DOPA to cell viability. Different letters mean significant difference between treated groups. One-way ANOVA post hoc Duncan (n=4; ± SD; $p < 0.05$).

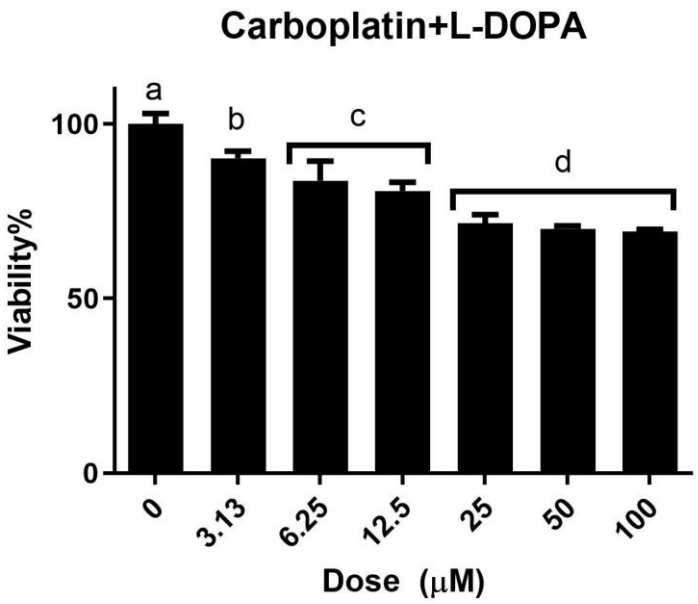

Figure 3. The effects of carboplatin, L-DOPA and combination of carboplatin and L-DOPA to cell viability for 24 hours. Different letters mean significant difference between treated groups. One-way ANOVA post hoc Duncan (n=4; ± SD; $p < 0.05$).

Conclusion

According to the study, the toxic effect of carboplatin chemotherapy drug on AML-12 cells decreased with L-DOPA application. L-DOPA appears promising regarding carboplatin-induced liver toxicity. More detailed studies are needed to evaluate the anticancer efficacy of the carboplatin/L-DOPA combination and its toxic effects on other tissues.

Acknowledgement

We would like to thank all staffs of Technology Research Development Application and Research Center of Trakya University for their kind support during this research.

REFERENCES

1. Livshits Z, Rao RB, Smith SWJEMC. An approach to chemotherapy-associated toxicity. 2014;32(1):167-203.
2. Ali MM, Aziz TAJA-RJoMS. Toxic Effect of Platinum Compounds: Molecular Mechanisms of Toxicity. 2021;1:81-8.
3. Podratz JL, Knight AM, Ta LE, Staff NP, Gass JM, Genelin K, et al. Cisplatin induced mitochondrial DNA damage in dorsal root ganglion neurons. 2011;41(3):661-8.
4. Desoize B, Madoulet CJCrioh. Particular aspects of platinum compounds used at present in cancer treatment. 2002;42(3):317-25.
5. Doz FJBdc. Carboplatin in pediatrics. 2000;87:25-9.

6. Chatterjee S, Rhee Y-H, Ahn J-C. Sulforaphene–carboplatin combination synergistically enhances apoptosis by disruption of mitochondrial membrane potential and cell cycle arrest in human non-small cell lung carcinoma. Journal of medicinal food. 2016;19(9):860-9.

7. Fuchs-Tarlovsky V. Role of antioxidants in cancer therapy. Nutrition. 2013;29(1):15-21.

8. Erisgin Z, Atasever M, Cetinkaya K, Dizakar SÖA, Omeroglu S, Sahin H. Protective effects of Nigella sativa oil against carboplatin-induced liver damage in rats. Biomedicine & Pharmacotherapy. 2019;110:742-7.

9. Alshorbaji MA, Dabdoub BR. Gum Arabic's Effect on Carboplatin-Induced Hepatotoxicity in Male Rats. NeuroQuantology. 2022;20(8):2547-56.

10. Carboplatin. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD)2012.

11. Özbek H, Uğraş S, Alıcı SJVTD. Rezene uçucu yağının carboplatin'e bağlı hepatotoksisite üzerine koruyucu etkisi. 2003;10(4):91-7.

12. Cao S, Wang C, Ma H, Yin R, Zhu M, Shen W, et al. Genome-wide Association Study on Platinum-induced Hepatotoxicity in Non-Small Cell Lung Cancer Patients. Sci Rep. 2015;5:11556. Epub 2015/06/24. doi: 10.1038/srep11556. PubMed PMID: 26100964; PubMed Central PMCID: PMCPMC4477405.

13. HASSAN ES, MAJEED SA, MOHAMMAD AR, GAEN KKJIJoPR. The protective effect of the N acetylcysteine on acute liver toxicity induced by carboplatin in rat model. International Journal of Pharmaceutical Research. 2019;11(3).

14. Zhou H, Tang L, Yang Y, Lin L, Dai J, Ge P, et al. Dopamine alleviated acute liver injury induced by lipopolysaccharide/d-galactosamine in mice. Int Immunopharmacol. 2018;61:249-55. Epub 2018/06/13. doi: 10.1016/j.intimp.2018.06.004. PubMed PMID: 29894864.

15. Sofian ZM, Shafee SS, Abdullah JM, Osman H, Razak SA. Evaluation of the Cytotoxicity of Levodopa and its Complex with Hydroxypropyl-ß-Cyclodextrin (HP-ß-CD) to an Astrocyte Cell Line. The Malaysian journal of medical sciences: MJMS. 2014;21(Spec Issue):6.

16. Dairman W, Christenson JG, Udenfriend S. Decrease in liver aromatic L-amino-acid decarboxylase produced by chronic administration of L-dopa. Proceedings of the National Academy of Sciences. 1971;68(9):2117-20.

17. Cotzias GC, Papavasiliou PS, Steck A, Duby S. Parkinsonism and levodopa. Clin Pharmacol Ther. 1971;12(2):319-22. Epub 1971/03/01. doi: 10.1002/cpt1971122part2319. PubMed PMID: 5577480.

18. Boukhris W, Kouassi E, Descotes J, Cordier G, Revillard J. Impaired T-dependent immune response in L-dopa treated BALB/c mice. Journal of clinical & laboratory immunology. 1987;23(4):185-9.

19. Sukmanskii OI, Arkhipenko VI, Cherkashina LP. [Role of adrenergic receptors in the mechanism of action of catecholamines and DOPA on proliferative processes]. Biull Eksp Biol Med. 1984;98(7):89-91. Epub 1984/07/01. PubMed PMID: 6147165.

20. Stewart DJ. Mechanisms of resistance to cisplatin and carboplatin. Crit Rev Oncol Hematol. 2007;63(1):12-31. Epub 2007/03/06. doi: 10.1016/j.critrevonc.2007.02.001. PubMed PMID: 17336087.

21. Türker NP, Bağcı U. Effect of Quinoa Plant on Metastasis and Ion Channels of Rat Brain Cancer Glioma Cell Lines. 2019.

22. Türker NP, Bağcı U, Onsekizoğlu-Bağcı P. Investigation of the Anticancer and Proliferative Effect of Broccoli Extract on Du145 Prostate Cancer and MEF Healthy Fibroblast Cell Lines. 2019.

23. Rodriguez JJ, Olabarria M, Chvatal A, Verkhratsky A. Astroglia in dementia and Alzheimer's disease. Cell Death Differ. 2009;16(3):378-85. Epub 2008/12/06. doi: 10.1038/cdd.2008.172. PubMed PMID: 19057621.

24. Abdel-Salam OM, Sleem AA, Shafee N. The dopamine agonist piribedil exerts hepatoprotective effects on carbon tetrachloride-induced hepatic damage. Comparative Clinical Pathology. 2013;22(3):413-9.

25. Mitchell IM, Pollock JC, Jamieson MP. Effects of dopamine on liver blood flow in children with congenital heart disease. The Annals of thoracic surgery. 1995;60(6):1741-4.