Avuloğlu Yılmaz, E., Toprak, Ş., Babacan, A. A. (2024). Examining the Proliferative Effect of Ozonated Olive Oil and Ozonated Distilled Water on Healthy Colon Fibroblast Cells and Colon Cancer Cells. *The Black Sea Journal of Sciences*, 14(1), 224-233.

The Black Sea Journal of Sciences, 14(1), 224-233, 2024. DOI: 10.31466/kfbd.1388212

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377 https://dergipark.org.tr/tr/pub/kfbd

Araştırma Makalesi / Research Article

Examining the Proliferative Effect of Ozonated Olive Oil and Ozonated Distilled Water on Healthy Colon Fibroblast Cells and Colon Cancer Cells

Ece AVULOĞLU YILMAZ[1*], Şenol TOPRAK[2], Aybüke Afra BABACAN[3]

Abstract

Ozone therapy is an alternative form of treatment based on the administration of ozone gas to the body in the treatment of diseases and different medical conditions, and ozone applications are recommended in some studies. In this study, it was aimed to determine the possible anti-cancer activity by enriching extra virgin olive oil and distilled water with ozone gas and determining its cytotoxic effect on colon cancer and normal colon fibroblast cells. The effect of ozone enriched extra virgin olive oil and distilled water on cell viability was determined by MTT assay on DLD1 (colon cancer) and CCD-18Co (healthy colon fibroblast) cell lines. In the DLD-1 cell line, ozonated distilled water and olive oil decreased *in vitro* cell viability at all concentrations and this decrease was most pronounced at higher concentrations (5 and 10 ppm). In the CCD-18Co cell line, ozonized distilled water and ozonized olive oil increased *in vitro* cell viability at all concentrations, but this increase was not significant compared to the control. The results of this study are consistent with the results of other studies in the literature. Therefore, ozone therapy is thought to be promising in cancer treatment.

Keywords: Ozone, MTT assay, Colon cancer, DLD1, CCD-18Co

Ozonlanmış Zeytinyağı ve Ozonlanmış Distile Suyun Sağlıklı Kolon Fibroblast Hücreleri ve Kolon Kanseri Hücreleri Üzerindeki Proliferatif Etkisinin İncelenmesi

Öz

Ozon terapi, hastalıkların ve farklı tıbbi durumların tedavisinde ozon gazının vücuda verilmesine dayanan alternatif bir tedavi şeklidir ve bazı çalışmalarda ozon uygulamaları önerilmektedir. Bu çalışmada, saf zeytinyağı ve distile suyun ozon gazı ile zenginleştirilerek olası anti-kanser aktivitesinin belirlenmesi ve kolon kanseri ve normal kolon fibroblast hücreleri üzerindeki sitotoksik etkisinin saptanması amaçlanmıştır. Ozonla zenginleştirilmiş saf zeytinyağı ve distile suyun hücre canlılığı üzerindeki etkisi DLD1 (kolon kanseri) ve CCD-18Co (sağlıklı kolon fibroblastı) hücre hatları üzerinde MTT testi ile belirlenmiştir. DLD-1 hücre hattında, ozonlanmış distile su ve zeytinyağı tüm konsantrasyonlarda *in vitro* hücre canlılığını azaltmıştır ve bu azalma en yüksek konsantrasyonlarda (5 ve 10 ppm) daha belirgindir. CCD-18Co hücre hattında, ozonlanmış distile su ve ozonlanmış zeytinyağı tüm konsantrasyonlarda *in vitro* hücre canlılığını artırmıştır, ancak bu artış kontrole kıyasla anlamlı değildir. Bu çalışmanın sonuçları literatürdeki diğer çalışmaların sonuçlarıyla uyumludur. Bu nedenle, ozon tedavisinin kanser tedavisinde umut verici olduğu düşünülmektedir.

Anahtar Kelimeler: Ozon, MTT testi, Kolon kanseri, DLD1, CCD-18Co

[1]Amasya University, Vocational School of Technical Sciences, Department of Health Information Systems, Amasya, Turkey, ece.yilmaz@amasya.edu.tr
[2]Amasya University, Vocational School of Technical Sciences, Department of Electronics and Automation, Amasya, Turkey, senol.toprak@amasya.edu.tr
[3]Amasya University, Sabuncuoğlu Şerefeddin Vocational School of Health Services, Department of Health Care Services, Amasya, Turkey, afra.babacan@amasya.edu.tr

[*]Sorumlu Yazar/Corresponding Author
Geliş/Received: 08.11.2023 **Kabul/Accepted:** 25.02.2024 **Yayın/Published:** 15.03.2024

1. Introduction

Cancer is one of the leading causes of death in the world and is caused by the uncontrolled proliferation of normal body cells. It can spread to surrounding tissues by clonal spread and throughout the body via blood or lymph. The most common types of fatal cancer are lung, breast and colon cancers (Minna et al., 2002; Özer et al., 2019). With the development of cancer, important changes occur within the cell. In the initial stage, cancer manifests itself with epigenome, chromosome and DNA damage that regulates gene expression. The initial phase is followed by a long process. With inflammation, genomically unstable cells grow. In the progression phase, as the cells proliferate, they further damage their genome. transforms into a malignant tumor. Due to their fast metabolism and impaired cellular signaling mechanism, cancer cells have high ROS production. High levels of ROS are generally harmful to cells and the redox state of cancer cells is different from that of normal cells (Çiftçi, 2017; Chung et al., 2020).

Ozone can directly damage the cell membrane through lipid peroxidation. This reaction leads to the formation of hydroperoxides, aldehydes, alkenes and lipoperoxides, which are partially reduced by antioxidant enzymes present in the inner layer of the cell membrane. Molecules that are not reduced react with cytoplasmic molecules such as NADPH. If there is not enough NADPH, they cause acute oxidative stress and damage to the cell, as can happen in cancer cells. In non-cancer cells, these molecules increase the synthesis of antioxidant molecules. Since cancer cells have an overloaded antioxidant system due to the increased level of ROS, they tend to further increase their antioxidant production. Therefore, non-cancerous cells can tolerate doses of ozone that are toxic to the cancer cell. On the other hand, there is a simultaneous increase in membrane permeability, which causes an apoptosis-inducing reaction in the ion concentration of the cytoplasmic fluid (Reuter et al., 2010; Lunov et al., 2014; Karabulut and Gülay, 2016; Glass et al., 2019; Chung et al., 2020).

Ozone (O_3) is a strong oxidizing agent that has been used for disinfection in water treatment for many years, especially in Europe. When ozone is added to water, it reacts with bacteria, viruses, and other organic and inorganic compounds, effectively destroying them, leaving no harmful by-products behind. This makes it an effective and environmentally friendly alternative to traditional disinfection methods such as chlorine. Ozone also has a number of other commercial applications. For example, it is used to disinfect bottled water and to keep swimming pools clean and free of harmful microorganisms. Ozone is also used in industrial processes to prevent contamination of cooling towers, which can increase efficiency and reduce maintenance costs. Disinfection By-Products (DBPs) are formed when chlorine-containing disinfectants react with organic chemical structures in water. Some DBPs, such as trihalomethanes (THM), have been associated with health-threatening problems such as an increased risk of cancer. Ozone is an alternative disinfectant that does not create

harmful by-products and chemical residues. Ozone oxidizes organic chemical structures in water, breaking them down into simpler, less harmful compounds that are not complex (Rubin, 2001; Rakness, 2011). Ozone can be created by a variety of processes, including natural and man-made sources.

Ozone is a thermodynamically unstable compound and can decompose to form oxygen without an external reaction triggering catalyst. The decomposition of the ozone molecule into the oxygen atom and molecule that forms it depends on factors such as temperature and pressure. At high temperatures and lower pressures, ozone molecules tend to decompose into oxygen molecules (Sonntag and Gunten, 2012). Ozone, also called trioxygen, has the chemical formula O_3. The structure of ozone is often portrayed as a resonant hybrid of two contributing structures: O=O-O ↔ O-O=O. In this resonance hybrid, the bond order of each oxygen-oxygen bond is 1.5. As a result, ozone gas dissolves in water. The corona discharge process is a method of producing ozone, using an electrical discharge to break down the oxygen molecule (O_2), then bringing the oxygen molecules together under high voltage to form ozone (O_3) (Stübinger et al., 2006; Toprak, 2021).

In recent publications, the preclinical hopeful findings of ozone gas on cancer cells was shown (Baeza-Noci and Pinto-Bonilla, 2021; Li et al., 2021). Therefore, in this study, olive oil and water, taken continuously into the human body, were enriched with different concentrations of ozone. Then, to determine their possible anticancer effect, their cytotoxicity in colon cells was examined by 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide (MTT) assay.

2. Materials and Methods

2.1. Test Compounds

The ozone generator at Amasya University Vocational School of Technical Sciences was used. To calculate 10 mg/m3 ozone, 1% O_3 and parts per million (ppm) values of ozone concentration by weight in the mixture. The formula to find 1% O_3 is C_1%-O_3 = O_3 concentration (mg/m^3) / (1% O_3 concentration (g/m^3) x 1000) x 100. Other concentrations to work with (1.25; 2.5; 5; 10 ppm) were determined by serial dilutions of the 10 ppm master stock concentration.

2.2. Cell Source

DLD-1 cell (ATCC® CCL221TM) and CCD-18Co cell (ATCC® CRL-1459TM) cell lines from the cell culture collection of Amasya University Central Research Laboratory were used.

2.3. Cell Culture

For the DLD-1 cell line, RPMI-1640 (Lot No RNBK9939 Sigma–Aldrich) medium containted with 10% FBS (Cat No 16000044 Gibco) Sodium pyruvate (1%) (Lot No 2051060 Biological Industries), Penicillin/streptomycin (1%) (Cat No P06-07100 Pan Biotech). EMEM (Cat No L0416 Biowest) medium was used for the CCD-18Co cell line. 250 mL of 1X EMEM medium used in cell culture studies was prepared with and 10% FBS, Penicillin/streptomycin (1%), and sodium pyruvate (1%). Cells were produced for 24 hours at 37 °C and 5% CO_2 and 95% humidity until MTT analysis.

2.4. MTT Assay

When the cells reached a sufficient number, necessary calculations were made for MTT analysis. Cells were added to the 96 plate ($5x10^3$ cells for each well) and incubated for 24 hours. On the second day, ozonated distilled water and ozonated pure olive oil (10; 5; 2.5; 1.25 ppm) were added to the plate at concentrations calculated as triplicate, and negative control and positive control (Mitomycin-C) concentrations were added to the plate. MTT dye is used to detect cell viability. MTT (20%) dye was prepared and incubated with cells in 96-well plates for 3 hours. Then MTT was removed from the wells and DMSO was added to each well. After approximately 10 minutes, absorbance values were determined spectrophotometrically with a Thermo Multiscan Go at 570 nm wavelength. One-Way ANOVA (Tukey test) was used to determine the change in absorbance values compared to the control. Percent cell viability was evaluated using Microsoft Excel and IC50 value (50% inhibitory concentration) was calculated by logarithmic slope plot.

3. Findings and Discussion

Cell viability was calculated after three different replicates obtained by MTT analysis. Values are expressed by the 50% inhibition concentration value (IC_{50}) measured by the linear range regression method. Distilled water and olive oil with ozone used in this study had antiproliferative effect in DLD-1 cancer cell line and they had no cytotoxic effect in CCD18-Co healthy colon cell line (Table 1 and 2).

Table 1. MTT absorbance measurements in ozonated distilled water and ozonated pure olive oil DLD-1 cell line

DLD-1 Cell line	Ozone with distilled water	Ozone with olive oil
	Mean±SE	Mean±SE
Control	1.04 ± 0.02	1.07 ± 0.08
Mitomycin-C	0.44 ± 0.07	0.61 ± 0.07
1.25 ppm	0.80 ± 0.05*	0.82 ± 0.06
2.5 ppm	0.79 ± 0.03*	0.88 ± 0.05
5 ppm	0.74 ± 0.01*	0.79 ± 0.05*
10 ppm	0.73 ± 0.01*	0.72 ± 0.02*

* Different from control $p < 0.05$ One-way ANOVA (Tukey test) SE: Standard Error

Accordingly in DLD-1 cell line ozonated distilled water was reduced on *in vitro* cell viability at all concentrations. Ozonated olive oil was also decreased cell viability at two highest concentrations (5 and 10 ppm). The percentage viability activities of ozonated distilled water in DLD-1 were 60.9% and 76.5%; percentage vitality activities in ozonated pure olive oil were determined between 66.9% and 81.7% (Figure 1 and 2). Therefore, ozonated distilled water and ozonated pure olive oil are thought to have antiproliferative activity in the DLD-1 cell line. When comparing ozonated distilled water and ozonated pure olive oil, it is seen that they show almost the same effect by looking at the percentage vitality values. Because both reduced the cancer cell population by half compared to the control. The 50% inhibitory concentration (IC_{50}) value was determined as 6.75 ppm in ozonated distilled water and 7.12 ppm in ozonated pure olive oil.

Table 2. MTT absorbance measurements in ozonated distilled water and ozonated pure olive oil CCD18-Co cell line

CCD18-Co Cell line	Ozone with distilled water	Ozone with olive oil
	Mean±SE	Mean±SE
Control	0.93 ± 0.02	1.05 ± 0.001
Mitomycin-C	0.41 ± 0.05	0.49 ± 0.005
1.25 ppm	1.12 ± 0.04	1.17 ± 0.06
2.5 ppm	1.11 ± 0.05	1.19 ± 0.07
5 ppm	1.07 ± 0.03	1.13 ± 0.03
10 ppm	1.06 ± 0.04	1.18 ± 0.03

SE: Standard Error

In CCD-18Co cell line, it was determined that ozonated distilled water and ozonated pure olive oil showed no activity on *in vitro* cell viability at all concentrations (Table 2). Therefore, it was observed that ozonated distilled water and ozonated pure olive oil had no cytotoxic effect in the CCD18-Co cell line (Figure 1 and 2).

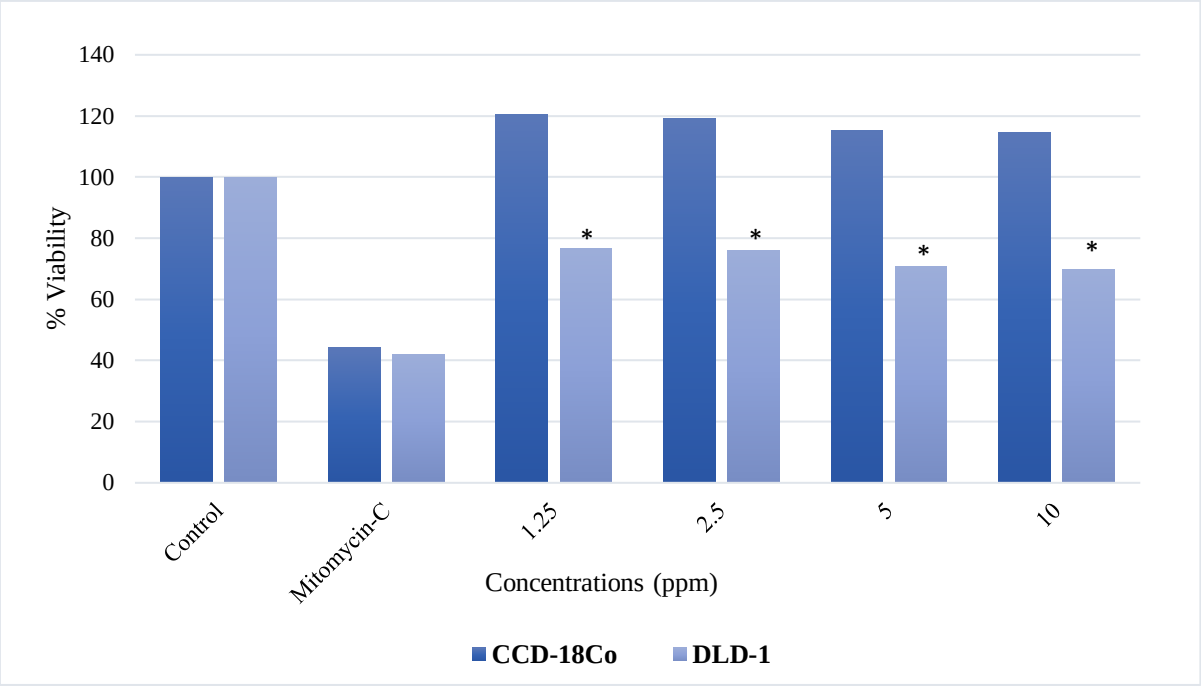

Figure 1. Percentage viability plot of ozonated distilled water on CCD18-Co and DLD-1 cell lines

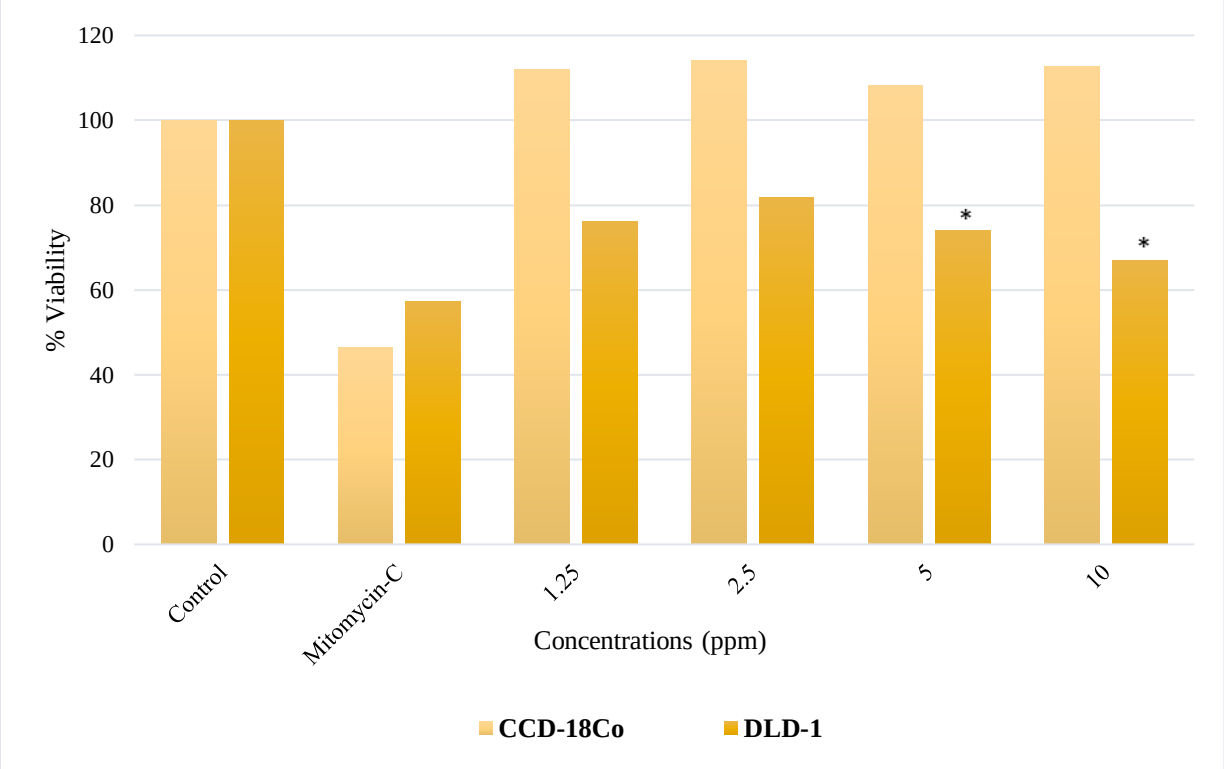

Figure 2. Percentage viability plot of ozonated virgin olive oil on CCD18-Co and DLD-1 cell lines

Cancer develops through the uncontrolled proliferation of normal body cells and causes significant changes within the cell as it develops. Chromosome and/or DNA damage present in early-stage cancer, together with defects in the regulation of gene expression, further damage the genome as cells proliferate. This damage can be caused by free radicals oxygen and nitrogen (Minna et al., 2002). Free radicals are generated by endogenous and exogenous sources. As a result of excessive production of ROS, a condition called "oxidative stress" occurs in cells (Çiftçi, 2017). The need to reduce such exogenous and endogenous increased risk factors in cancer and to investigate new therapies is evident. Through early diagnosis, it can be understood how the best health technology and new treatments can affect cancer. While surgery, radiotherapy, chemotherapy or a combination of both is still the norm for many types of cancer, new complementary and alternative treatments such as immunotherapy are showing promising results (Halliwell, 2006). Ozone therapy can also be classified among these new therapies. According to a study conducted in 2017, HT-29 cell line were incubated with different concentrations of ozone ranging from 10 to 50 μg/ml at alone or in combination with Cisplatin and 5-Fluorouracil. Anti-inflammatory studies have also been performed by incubating HT-29 with or without 20, 30, or 50 μg/ml ozone, with MTT method. Ozone alone appeared to have a time- and concentration-dependent cytotoxicity against HT-29 cell line (IC_{50}: 30 μg/ml). The combination of ozone with cisplatin and 5-fluorouracil increased the cytotoxicity by 15-20%. These results showed that ozone may be beneficial in the treatment of HT-29 colon cancer in with 5-fluorouracil and Cisplatin, with significant inhibition of cytokines. Cytokines have a central role in colon cancer cell survival and chemoresistance (Simonetti et al., 2017). In another study designed to evaluate the therapeutic effects of ozonated olive oil (OzOO) on acute radiation proctitis. The rats were divided into three groups. Control group, radiation irradiation with saline group, and radiation irradiation with OzOO group. The treatment lasted for ten days and at the end of the period, a decrease in significant pathological changes induced by radiation was detected in the animals in the OzOO irradiation group (Gültekin et al., 2013). In different work, the therapeutic effect of radiotherapy and ozone combination, which has the ability to increase the destruction of cancer cells by increasing the oxygen level in the body. It was examined on advanced tongue cancer in rats. A total of 36 female rats were included in this study. At the end of the 20th week, some groups of rats were sacrificed. Others were waited until oral food intake was stopped. The necessary applications were made and the survivals were evaluated. In this study, it has been shown that radiotherapy plus ozone application both provide histopathological improvement and prolong survival in a rat model with advanced tongue cancer (Dogan et al., 2018).

Both the safeness and possible anti-cancer effect of ozonated water were investigated using mice with tumors and normal controls. Ozonated water (20.8, 41.6, 104 and 208 mM) was given

directly to the tumor and was found to inhibit tumor growth by causing necrosis in the tissue. In addition, ozonated water did not affect normal tissue. These results indicate that ozonated water selectively induces necrosis only in tumor cells (Kuroda et al., 2015). Ozone, at the right dosage, appears to maintain normal cellular action without damaging healthy cells because the antioxidant system in these cells can usually overcome this damage, whereas the antioxidant capacity of cancer cells is virtually exhausted. Ozone has been reported to have a radiosensitizing effect when used in vitro with some radiotherapy protocols, rendering radioresistant tumor cells radiosensitive. In addition, the biochemical pathways used by ROS to induce cell apoptosis in cancer cells have been described. Accordingly, ozone increases ROS in tumor cells, causing cancer cell damage similar to that produced by cancer drugs (Mokhtari et al., 2019; Li et al., 2021). When chemotherapy drugs and ozone are used together, an enhanced or synergistic effect has been reported, similar to that observed in radiotherapy protocols (Zänker and Kroczek, 1990). However, in another study, this synergistic effect was investigated again and it was reported that it varied according to the various chemotherapy drugs used and may have different effects on various cancer cell lines (Cannizzaro et al., 2007).

4. Conclusions and Recommendations

In recent years, articles have been published supporting the *in vivo* and *in vitro* role of ozone (O3), which remains popular in scientific studies, in directly inducing cancer cell damage in a harmless way for non-cancer cells. The *in vivo* and *in vitro* studies have positively affected our work and advanced the ozone view we advocate in cancer treatment. Ozone has a wide variety of medical applications mainly due to its powerful oxidative properties. On the other hand, a few clinical papers were published so it is important to remember that ozonated products should be used under the supervision of a medical or health professional and that there may be potential risks and side effects.

The use of ozone in cancer requires a great deal of *in vivo* and *in vitro* research, including testing of a large number of cancer cell lines with different concentrations of ozone and ozonized products. Ozone application is thought to be promising in the treatment of cancer.

Acknowledgements

Author wishes to thank Dr. Ceren BAŞKAN for her support.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The authors declare that all the rules required to be followed within the scope of "Higher Education Institutions Scientific Research and Publication Ethics Directive" have been complied with in all processes of the article, that The Black Sea Journal of Science and the editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than The Black Sea Journal of Science.

Ethical approval is not required for this research article.

References

Baeza-Noci, J., and Pinto-Bonilla, R. (2021). Systemic review: ozone: A potential new chemotherapy. *International Journal of Molecular Sciences*, 22(21), 11796.

Cannizzaro, A., Falzacappa, C. V., Martinelli, M., Misiti, S., Brunetti, E., and Bucci, B. (2007). O2/3 exposure inhibits cell progression affecting cyclin B1/cdk1 activity in SK-N-SH while induces apoptosis in SK-N-DZ neuroblastoma cells. *Journal of Cellular Physiology*, 213(1), 115-125.

Chung, L. Y., Tang, S. J., Wu, Y. C., Yang, K. C., Huang, H. J., Sun, G. H., and Sun, K. H. (2020). Platinum-based combination chemotherapy triggers cancer cell death through induction of BNIP3 and ROS, but not autophagy. *Journal of Cellular and Molecular Medicine*, 24(2), 1993-2003.

Çiftçi, N. (2017). Oksidatif stresin kanserdeki rolü: Antioksidanlar kanser progresyonunun yakıtı olabilir mi. *Ahi Evran Tıp Dergisi*, 1, 8-13.

Dogan, R., Hafız, A. M., Kiziltan, H. S., Yenigun, A., Buyukpinarbaslili, N., Eris, A. H., and Ozturan, O. (2018). Effectiveness of radiotherapy+ ozone on tumoral tissue and survival in tongue cancer rat model. *Auris Nasus Larynx*, 45(1), 128-134.

Glass, S. B., Gonzalez-Fajardo, L., Beringhs, A. O. R., and Lu, X. (2019). Redox potential and ROS-mediated nanomedicines for improving cancer therapy. *Antioxidants & Redox Signaling*, 30(5), 747-761.

Gültekin, F. A., Bakkal, B. H., Sümer, D., Köktürk, F., & Bektaş, S. (2013). Effects of ozonated olive oil on acute radiation proctitis in rats. *Balkan Medical Journal*, 2013(4), 369-374.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now?. *Journal of Neurochemistry*, 97(6), 1634-1658.

Karabulut, H., and Gülay, M. Ş. (2016). Serbest radikaller. *Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi*, 4(1), 50-59.

Kuroda, K., Azuma, K., Mori, T., Kawamoto, K., Murahata, Y., Tsuka, T., Osaki, T., Ito, N., Imagawa, T., Itoh, F., and Okamoto, Y. (2015). The safety and anti-tumor effects of ozonated water in vivo. *International Journal of Molecular Sciences*, 16(10), 25108-25120.

Li, J., Zeng, T., Tang, S., Zhong, M., Huang, Q., Li, X., and He, X. (2021). Medical ozone induces proliferation and migration inhibition through ROS accumulation and PI3K/AKT/NF-κB suppression in human liver cancer cells in vitro. *Clinical and Translational Oncology*, 23, 1847-1856.

Lunov, O., Zablotskii, V., Churpita, O., Chánová, E., Syková, E., Dejneka, A., and Kubinová, Š. (2014). Cell death induced by ozone and various non-thermal plasmas: therapeutic perspectives and limitations. *Scientific Reports*, 4(1), 7129.

Minna, J. D., Roth, J. A., and Gazdar, A. F. (2002). Focus on lung cancer. *Cancer Cell*, 1(1), 49-52.

Mokhtari, H., Farahmand, L., Yaserian, K., Jalili, N., and Majidzadeh-A, K. (2019). The antiproliferative effects of cold atmospheric plasma-activated media on different cancer cell lines, the implication of ozone as a possible underlying mechanism. *Journal of Cellular Physiology*, 234(5), 6778-6782.

Özer, Ö. F., Güler, E. M., Selek, Ş., Çoban, G., Türk, H. M., and Koçyiğit, A. (2019). Akciğer, meme ve kolon kanserli hastalarda oksidatif stres parametrelerinin değişimi. *Harran Üniversitesi Tıp Fakültesi Dergisi*, 16(2), 235-240.

Rakness, K. L. (2011). *Ozone in drinking water treatment: process design, operation, and optimization*. Denver, USA: American Water Works Association.

Reuter, S., Gupta, S. C., Chaturvedi, M. M., and Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: how are they linked?. *Free Radical Biology and Medicine*, 49(11), 1603-1616.

Rubin, M. B. (2001). The history of ozone. The Schönbein period, 1839–1868. *Bulletin for the History of Chemistry*, 26(1), 40-56.

Simonetti, V., Quagliariello, V., Giustetto, P., Franzini, M., and Iaffaioli, R. V. (2017). Association of ozone with 5-fluorouracil and cisplatin in regulation of human colon cancer cell viability: in vitro anti-inflammatory properties of ozone in colon cancer cells exposed to lipopolysaccharides. *Evidence-Based Complementary and Alternative Medicine*, 7414083.

Sonntag, C., and Gunten, U. (2012). *Chemistry of ozone in water and wastewater treatment*. London, UK: IWA Publishing.

Stübinger, S., Sader, R., and Filippi, A. (2006). The use of ozone in dentistry and maxillofacial surgery: a review. *Quintessence International*, 37(5), 353-359.

Toprak, Ş. (2021). Ozone generator and ozone generation. *Journal of Amasya University the Institute of Sciences and Technology*, 2(2), 16-25.

Zänker, K. S., and Kroczek, R. (1990). In vitro synergistic activity of 5-fluorouracil with low-dose ozone against a chemoresistant tumor cell line and fresh human tumor cells. *Chemotherapy*, 36(2), 147-154.